ELEMENTARY EXERCISES

IN

PHYSIOLOGY

BY

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SECOND EDITION   REVISED AND ENLARGED

PREFACE TO THE SECOND EDITION

The present manual represents a second edition of the former Practical Exercises in Comparative Physiology and Urine Analysis.

A separate course is now devoted to urinary examinations and the directions therefor are issued separately. To replace this, more experimental work has been added, and the experiments in chemical physiology have been augmented.

It is hoped that the following experiments may be of use to the student in training the power of observation ; cultivating skill and accuracy ; and emphasizing the fact that first hand knowledge is the most desirable.

I am indebted to my assistants, past and present, for useful suggestions in connection with the course.

FEBRUARY, 1906.   P. A. F.

PREFACE TO THE FIRST EDITION

This little manual has been designed, especially, to meet the needs of those students who desire to become physicians or teachers of science. While not intended as an exhaustive treatise, it has been the endeavor to concentrate a number of useful experiments into a small compass. For a few of these experiments a somewhat special equipment may be needed ; but the majority of them may be as easily performed in a preparatory school as in a college, with a little experience and ingenuity on the part of the instructor.

It has been the aim to explain clearly the essential steps of the experiments and the reasons for them ; but, at the same time, to leave opportunities for observation on the part of the students themselves, and to have them record their own inferences of the phenomena observed.

In the preparation of this laboratory guide, the standard and recent books and papers bearing on practical physiology have been largely drawn upon, among which may be mentioned the works of Stirling, Halliburton, Waller, Brunton, Foster and Langley, Gamgee, Stewart, Long, Hall, Chase and others.

In a sense, all physiology is comparative since we are dependent upon the lower forms of animal life for much of our knowledge of the function of similar organs and parts in the higher ; but there are differences of function in the different genera of animals, correlated with differences in structure and mode of life. Wherever it has been practicable, in the text, these differences have been pointed out.

AUGUST, 1898.   P. A. F.

SECTION ON B-B. ENDS.

TABLE FOR PHYSIOLOGICAL LABORATORY
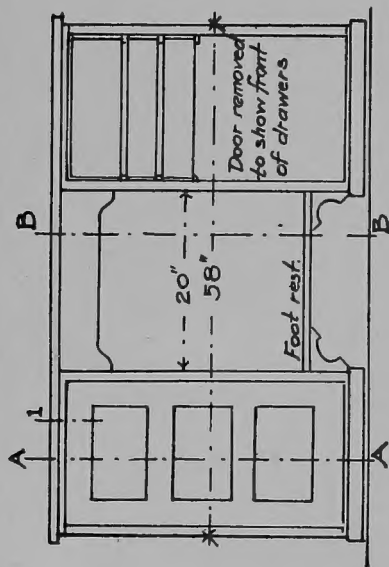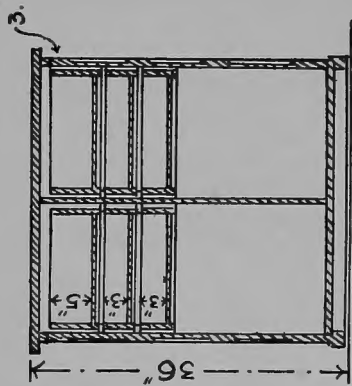
A Combined Locker and Laboratory Table

Specifications.   Both sides of the table are to be exactly alike. Each table will then have four doors, four drawers each five inches deep in the clear, and eight drawers each three inches deep in the clear.

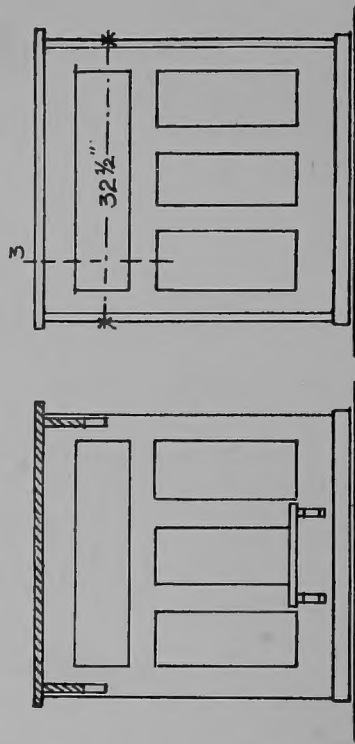Exterior of tables and fronts of drawers are to be of selected red oak ; drawer guides or slides of oak maple or cherry, and balance of interior work of poplar.

Each door shall be hung with one pair good brass fast pin butts, and shall be fitted with an " Anti-dial " combination lock. Each table shall be fitted with eight " standard " No. 7, all steel castors.

Except the top, all exposed work, including drawer fronts, shall be filled with silica paste filler, and shall then be finished with one coat of white shellac and one coat of Johnson's, or equally good wax.   Inside and drawers, except fronts, shall have one coat of orange shellac.

The table in question was designed for laboratory work in Physiology and Materia Medica.   The height and also the area of the table top is somewhat greater than ordinary for the reason that in experimental physiology it is necessary at times to have considerable apparatus upon the table, and the height is desirable because in some experiments the student can do his work better standing than sitting.   The foot rest attached to the tables, in connection with a stool a trifle higher than usual (24 inches), enables the table to be perfectly serviceable and entirely satisfactory for all forms of work at which it is desirable that the student should sit.

The chief advantage of the table, however, is believed to rest upon the fact that a considerable economy of space and convenience to the worker is subserved.   The floor space covered by the table, in many instances, is not utilized at all, except for the work done upon the top of the table.   Lockers, when necessary, have been built along the walls of the laboratory or in the hallway, or in an adjoining room, thus taking up space which might be profitably utilized by wall cases containing specimens, models, or general apparatus bearing upon the laboratory course.   Students often pass to and fro from table to locker, causing more or less jar and vibration, especially annoying if microscopical work is

going on. Such an arrangement is doubly inconvenient. It is annoying to the student to be obliged to go from table to locker. It is also annoying to his fellow workers to have him do so.

The combined lockers and table obviates these disadvantages. Each table contains four lockers and two students can work at one table and have their apparatus right at hand. Twelve tables will provide lockers for forty-eight students and twenty-four students can work at the tables at one time.

The table would appear to be useful for biological work in general, although in certain cases a proportionate change in dimensions may be desirable.

The cost of the combined locker-table is less than the total cost of a table and four lockers built separately. The combined locker-table, including a combination lock for each locker, can be built in red oak for fifteen or sixteen dollars each.

PART I

Chemical Physiology

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PART II

CHEMICAL PHYSIOLOGY

1 dozen test tubes, 6 inch

1 dozen test tubes, 5 inch

1 Minim pipette

1 Beaker, 10 oz.

1 Graduate, 30cc

1 Graduate, 250cc

1 Flask

1 Funnel, 1½ inch

1 Funnel, 3 inch

1 Watch glass

1 Evaporating dish, 8 oz.

1 Urinometer

1 Glass rod

1 Dialyzer

1 Thermometer

1 Crucible, 8cc

1 Piece wire gauze

1 Piece absorbent cotton

1 Box matches

1 Test tube brush

1 Test tube rack

1 Test tube holder, wire

1 Tripod

1 Piece muslin

1 Pack filter papers, 3 inch

1 Pack filter papers, 6 inch

1 Sponge

1 Clay triangle

2 Tin cans

1 Copper water bath

1 Towel

Special apparatus, not found in the locker, may be obtained, when needed, by handing an order for it to one of the assistants.

EXPERIMENTAL PHYSIOLOGY

APPARATUS

1 Metal tray
1 Frog board with 4 clips
1 Wooden stand
1 Iron standard with 2 clamps
1 Femur clamp
1 Muscle lever
1 Strip of pins
5 Coils of wire
5 Sheets Kymograph paper
   Saline solution

   Battery
   Induction coil
   Make and break key
   Short circuiting key
   Electrodes
   Kymograph

Special apparatus, not found in the locker, may be obtained, when needed, by handing an order for it to one of the assistants.

It is desirable that the physiologic work should be correlated so far as possible, *i. e.* the lectures, recitations and laboratory work should be given during the same term and have as close connection with each other as possible. If this is not practicable then the recitations or lectures should precede the work in the laboratory.

Quizzes on the work gone over may be regarded as a part of the laboratory course and should occur at frequent intervals.

Notes should be taken on the experiments as soon as they are performed. The manual is intended as a laboratory companion and the blank pages are provided for this purpose. Note taking is a portion of the laboratory work and the notes are to be submitted to the instructors for correction.

Unexcused absences, tardiness in beginning the work and leaving without permission before the end of the period will be noted, and the mark or standing of those concerned will be correspondingly reduced at the end of the term.

Many of the reagents which are to be frequently used will be placed on the student's desk on or before the periods in which they are needed, other reagents less frequently used, will be placed on the general shelves and the student *must not* carry these general reagents to his desk.

Any special reagent or apparatus, not included in the above, may be obtained when needed, by handing an order for it to one of the assistants.

Before beginning his experiments, the student should inventory his locker and check off on the slip, furnished for the purpose, each piece of apparatus that is designated. The slip is then signed by the student and is held by the department as a receipt. At the end of the term the contents of each locker are examined and compared with the receipt. Any articles that are missing, broken or damaged are charged to the student.

Where there is any doubt as to the result of the experiment or any indefiniteness in the reaction consult with the assistant before taking up a new experiment.

PROTEINS

1. The proteins form the chief organic constituents of the animal body, and occur in greater or less quantity in plants. Their composition is very complex and but little is known of their structure. All proteins contain carbon, hydrogen, oxygen and nitrogen, most of them also contain sulphur, several phosphorus, and some, iron.

Proteins are almost all amorphous, non-volatile, non-diffusible, colorless, odorless and nearly tasteless solids. They vary in solubility. When burned or subject to dry distillation, they give off a disagreeable odor due to ammoniacal derivatives. Proteins are also distinguished by the ease with which they undergo chemical change under the influence of reagents, ferments, or variations in temperature. They all undergo the process of putrefaction. By boiling with dilute acids or alkalies, and also by the action of certain ferments, the proteins undergo hydrolysis, forming simpler compounds.

Classification. Because of incomplete knowledge of their structure, an accurate classification is difficult. The simplest method, perhaps, is according to their source. 1. *Native proteids*, which may be isolated from the organism without loss of their properties. 2. *Derived proteids*, which are obtained by the action of heat and reagents on native proteids.

A classification more commonly in use, however, is according to their composition. 1. *Simple proteids.* 2. *Compound proteids.* 3. *Albuminoids.*

Simple proteids are the most prominent solid constituent in muscle, glands, and blood serum, and to a greater or less extent in *all* tissues. The average percentage composition of simple proteids is: carbon, 50%–55% ; hydrogen, 6.3%–7.3% ; nitrogen, 15%–18%; oxygen, 21%–24%; sulphur, 0.3%–2.5%; Some contain phosphorus, 0.85%, and a few a trace of iron.

By the action of heat or certain reagents, soluble simple proteids become insoluble modifications by *coagulation*. A coagu-

lated proteid cannot return to its original condition, thereby differing from a precipitated proteid. The temperature at which coagulation occurs, depends upon the nature of the proteid present, the reaction of the solution, and the presence of neutral salts—*e. g.* an alkaline solution does not coagulate on boiling, a neutral solution will do so partially, an acid solution completely coagulates, provided the quantity of neutral salts present is not too small.

Albumins are soluble in water, coagulated by heat, and precipitated by saturating their solution with ammonium sulphate, and include ovalbumin (white of egg), serum albumin of blood serum and serous fluids, lactalbumin of milk, and myo-albumin of muscle.

In working through the experiments, a good general rule to follow—unless otherwise directed—is not to use the whole of the material or solution at once but only a small portion of it ; so that other tests may be tried if necessary.

2. Preparation of Egg-Albumin Solution. Break a small hole in the end of a fresh egg ; carefully pour out 10 cc. of the white of the egg into a beaker. Let the yolk remain in the shell and reserve for later use. Add about 200 cc. of distilled water to the beaker containing the egg-white. Stir thoroughly with a glass rod to break up the membranes and thus liberate the albumin. Filter through a piece of muslin. Any opalescence is due to the precipitation of globulins. Egg-white contains about 11%–12% of egg-albumin, together with small quantities of globulins, grape-sugar, and mineral matter. The white of one egg will serve for a number of students.

A good solution for laboratory use may also be prepared by dissolving 1 gram of dry albumin in 200 cc. of distilled water.

3. Heat 5 cc. of the albumin solution in a test-tube to boiling. Notice the coagulation. Add a little nitric acid, the coagulum may turn yellow but it does not dissolve.

4. Xanthoproteic reaction. To a little of the albumin solution in a test-tube, add some strong nitric acid ; a precipitate is formed, white in color, which on being boiled, turns yellow. After cooling, add ammonia till alkaline ; the yellow color changes to orange. With weak solutions there may be no precipitate at all. If only traces of albumin are present, the yellow color with the nitric acid may fail to appear, but

3. Albumin solution + heat = white coagulation. This + nitric acid = yellow coagulation or rather white turns yellow. ~~with a white precipitate.~~

4. Albumin solution + strong nitric acid causes a white precipitate to be formed which on being boiled turns yellow. After this has been allowed to cool I added ammonia till alkaline; the color changed to orange

If the above colors are present when above re ————————————— ~~show that~~ albumin is present

the addition of ammonia gives the final test with, perhaps, a yellow instead of an orange color.

5. To another portion of the solution add some of Millon's reagent ; a white precipitate is formed, which on boiling, becomes brick-red in color.

6. Piowtrowski's reaction, (also known as the biuret reaction). Add excess of strong solution of caustic potash and then a drop or two of very dilute solution of cupric sulphate, when a violet color results. The reaction occurs more quickly if heat is applied, and the color deepens. Make a check test by using some water instead of the albumin solution. (Peptones and albumoses give a pink color when only a trace of copper sulphate is used.)

7. Adamkiewicz's reaction. Dissolve a small quantity of the proteid by boiling with glacial acetic acid, cool and hold the test tube in an inclined position ; allow 2 cc. of concentrated sulphuric acid to flow down the side : a violet or purple color develops where the liquids meet.

8. Acidify another portion strongly with acetic acid and add a few drops of potassium ferrocyanide. A white precipitate is obtained. Peptones do not give this reaction. (Albumin is also precipitated by lead acetate, mercuric chloride ; picric acid ; strong acid, e. g., nitric; tannin ; and strong alcohol).

9. Make some of the albumin solution strongly acid with acetic acid, add a few crystals of sodium sulphate and boil. All proteids except peptones are precipitated in this manner. The filtrate, after boiling, can be used for other tests, (peptones) as the acid and sulphate do not decompose the solution.

10. Indiffusibility of Albumin. Place some of the solution in a dialyzer. The salts (crystalloids) diffuse readily. At the next exercise test for chlorides by adding a little silver nitrate solution to a portion of the diffusate. Apply to another portion of the diffusate any of the preceding tests for albumin. None will be found. Albumin belongs to the group of colloid bodies.

11. Globulins are proteids insoluble in water, but are soluble in dilute saline solutions. They are coagulated by heat and are precipitated by saturating their solution with magnesium sulphate or sodium chloride, and by the addition

5 To a portion of albumin solution a small amount of Millon's reagent was added; a white precipitate was formed, which on boiling, becomes brick-red in color. ⎯

6. Piotrowski's reaction.

To a solution of albumin an excess of strong solution of cupric sulphate was added which changed the solution to a violet color. When heated the color deepens. A check test was made by using H_2O instead albumin solution. When H_2O was used the color was not as deep a color.

8 To a solution of albumin strongly acified with acetic acid a little potassium ferrocyanide solution was added. A white precipitate is obtained.

9. A solution of albumin was made strongly acid with acetic acid and few crystals of sodium sulphate and then boiled.

The solution was quite clear except a white cloudy precipitate was seen he⎯

of an equal volume of saturated solution of ammonium sulphate. They comprise (*a*) serum-globulin of blood plasma, lactoglobulin of milk, myoglobulin, myosin, and musculin of muscle ; (*b*) myosinogen of muscle, a peculiar proteid, having properties like both albumins and globulins ; (*c*) fibrinogen, differing from other globulins in being precipitated from its solution by an equal volume of saturated solution of sodium chloride and by forming fibrin when acted on by the fibrin ferment ; and (*d*) vitellin differs from globulin in not being "salted out" by sodium chloride. Ovavitellin of egg-yolk and crystallin of the lens of the eye are vitellins.

12. Take a small portion of the yolk in a test tube and shake thoroughly with ⅓ tube of ether ! ! for several minutes. Let settle. Carefully pour off the ether into evaporating dish and repeat several times till residue is colorless or nearly so.

(*a*). Let the substance in the dish evaporate till the odor of ether is all gone. What is the substance? Pour a little of it in water and note results, also after placing a drop on a piece of paper.

(*b*). Transfer the residue in test tube to watch glass and let dry thoroughly. Divide into two portions. Place portion (1) in a test tube, fill tube ⅓ full of water and shake thoroughly for five or ten minutes. Does the substance dissolve ? Let settle, pour liquid through " filter " and test it for proteid with the biuret test. Save this test and compare with next.

To the undissolved residue in the tube add a little 10% solution of sodium chloride and shake as before. Result? Filter and apply the biuret test. Result ? Conclusions ?

13. Place portion (2) in crucible, add a few drops of concentrated HNO_3 and carefully evaporate to dryness high over the flame, charring as little as possible. When dry, let cool. Add a few drops of concentrated HNO_3 again and repeat evaporation as above. Now gradually lower crucible into flame and heat until the most of the black substance disappears. Let cool, add a little concentrated HCl and heat to boiling. Dilute with an equal volume of water and filter. Divide into four portions. To the first add some potassium ferrocyanide solution, and to the second some potassium sulphocyanide solution. A blue color in the former and a red color in the latter indicates iron.

12. A small portion of egg yolk was taken and about 1/3 test tube of either was added to it. This was shaken thoroughly for several minuets and allowed to settle. The ether was now allowed to evaporate from an evaporating dish. This was repeated until the residue was nearly colorless.

(a) The substance was allowed to stand into evaporating dish until the odor of ether was all gone. This substance is fat. A little of this substance was put into H_2O, and the H_2O became greasy. A little of this substance was put on paper it also showed greasy properties

(b) The residue in evaporating dish was transferred to watch glass and allowed to thoroughly evaporate. A portion of this was placed into a test tube filled with 1/3 H_2O and shaken thoroughly for 5 minuets. The substance dissolves. After shaking it was allowed to settle, evapor filtered. and tested for protein with biuret test. Test was positive.

To the residue left in test tube was added a little 10% solution of _____ and the biuret test applied. _____ egg was contain protein fat

To the third portion add a few drops of barium chloride and let stand. A white ppt. indicate sulphates.

To the fourth add a few drops of conc. HNO_3 and an equal volume of ammonium molybdate solution. Let stand. A yellow crystalline ppt. indicates phosphates.

14. *Peptones.* Peptones are proteids soluble in water, but not coagulable by heat. They are the result of proteid digestion and are diffusible through animal membranes. Albumoses or proteoses are substances intermediate in constitution between albumins and peptones.

15. Peptones differ from albumins as follows : They are not coagulated by heat ; they are not precipitated by adding sodium chloride ; they are not precipitated by acids or alkalies; they are not precipitated by sodium sulphate ; they are not precipitated by potassium ferrocyanide ; they yield a pink color with Piowtrowski's test instead of a violet as given for albumin. Make a solution of peptone and apply Piowtrowski's test. (See plate I).

16. Like albumin they are precipitated by the addition of tannic acid ; they are also precipitated by alcohol, but it must be remembered that all proteids are precipitated by alcohol, and that the absence of other proteids must be proved before deciding that the precipitate with alcohol is peptone.

II

DERIVED PROTEIDS. COMPOUND PROTEIDS. ALBUMINOIDS.

17. *Acid and Alkali Albumins or Albuminates* are derived proteids. Alkali albuminates are obtained by the action of alkalies on native proteids to such an extent that nitrogen and occasionally sulphur also are eliminated from the molecule. The change takes place slowly at the ordinary temperature, more rapidly on heating.

Acid albuminates are obtained by digesting native proteids with dilute acids.

These albuminates are insoluble in water and in neutral salt solution, but easily soluble in the presence of a small

1 ———————————————— followed as given.

1 Test Tube Potassium ferrocyanide was added color became blue
2 Test " " sulphocyanide " " " red
3 Test " Barium chloride . . . . white
4 Test " HNO_3 + Ammonium molybdate was added turned yellow

conclusion Iron, sulphates and phosphates
are found in albumin.

15 Some peptone was taken and
the test of Piowtrowski's was applied
the solution became pinkish while albumin
was violet.

10 White precipitate was formed when
silver nitrate was added.

amount of either acid or alkali. The solution is not coagulated by heat. The albuminate is completely precipitated when the solution is neutralized. A solution in dilute acid is completely precipitated by saturation with ammonium sulphate or sodium chloride, while the solution in alkali is not precipitated by similar treatment.

Syntonin, formed during gastric digestion, is an important example of acid albumin or albuminate.

18. *Albuminates.*—Action of acids and alkalies on albumin. Take three test-tubes and label them A, B, C. In each, place an equal amount of diluted egg-white, like that used at the last exercise. To A add a few drops of 0.1% solution of caustic potash. To B add the same amount of 0.1% solution of caustic potash. To C add a rather larger amount of 0.2% hydrochloric acid, (6.5 cc. concentrated acid to 1 liter of water).

Put all three into a warm water bath at about the temperature of the body (36–40 C).

19. After ten or fifteen minutes remove test tube A and boil. The proteid is no longer coagulated by heat, having been converted into *alkali-albumin*. After cooling, color with litmus solution, and neutralize with 0.2% acid by the contact method. At the neutral point a precipitate is formed, which is soluble in excess of either acid or alkali. Quite as delicate a test may be obtained without the litmus by means of the contact method. A distinct white precipitate appears between the two layers of fluid.

20. Next remove B. This also now contains alkali-albumin. Add to it a few drops of a sodium phosphate solution, color with litmus, and neutralize as before. Note that the alkali-albumin now requires more acid for its precipitation than in A, the acid which is first added converting the sodium phosphate into acid sodium phosphate.

21. Now remove C from the bath. Boil it. Again there is no coagulation, the proteid having been converted into *acid-albuminum* or *syntonin*. After cooling color with litmus and neutralize with 0.1% alkali. At the neutral point a precipitate is formed soluble in excess of acid or alkali. (Acid-albumin is formed more slowly than alkali-albumin, so that it is well to take plenty of time).
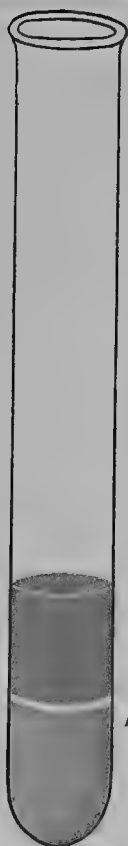
acid-albumin. ppt.   alkali-albumin. ppt.   peptone.

... test tube was ...... as directed and put in 38-40°
of H_2O. After 12 min Test tube A was taken out and
~~allowed to cool~~ boiled, it did not coagulate. After
cooling it was colored with litmus solution &
neutralized with .2% acid by the contact method
a precipitate formed at the neutral point.

20 To B was added a few drops of sodium
phosphate and colored with litmus, then neutralized
more acid was used in B than A.
  a white precipitate was formed.

21 C was boiled but it did not coagulate
after cooling it was colored ~~and~~ neutralized with litmus.
at the neutral pt a precipitate was formed.

22. *Metallic albuminates.* Add to separate tubes of albumin solution, a crystal each of copper sulphate, silver nitrate and a small amount of mercuric chloride. In each of the three tubes metallic albuminates will be precipitated.

23. *Coagulated proteids* are obtained by the action of heat, enzymes, acids, and other reagents on native proteids, by a process of unknown nature, and have been found in the liver and other glands. Fibrin is a coagulated proteid formed by the action of the fibrin ferment on the fibrinogen of blood plasma.

24. *Proteoses or Albumoses.* Proteoses are the products of the hydrolysis of proteids. They are important intermediate products in the digestion of proteids in the animal body, are soluble in water, not coagulated by heat, and are precipitated by saturating their solution with ammonium sulphate.

25. **Compound proteids** on hydrolysis yield as products of the first splitting a simple proteid and some non-proteid substances. They are subdivided, according to this non-proteid result, as hemoglobins, glycoproteids, and nucleoproteids.

26. *Hemoglobins* on hydrolysis yield a simple proteid and hematin. Hemoglobin is the coloring agent of the blood and enters into combination with certain gases—for instance, carbon dioxide, nitrogen dioxide, and hydrocyanic acid—more readily than with oxygen, and the poisonous properties of these gases are due largely to their power of satisfying the affinities of the hemoglobin, and in this way rendering it incapable of taking up oxygen.

Hemoglobin is soluble in water, in dilute solutions of albumin, of the alkalies and their carbonates, and in sodium or ammonium phosphate. It is insoluble in strong alcohol, ether, and in the volatile and fatty oils. With the spectroscope both oxyhemoglobin and reduced hemoglobin show characteristic absorption bands. Hemoglobin crystals may be obtained, which differ in shape and solubility in water according to the species of animal from which the blood is obtained.

27. *Glycoproteids* yield a substance capable of reducing an alkaline solution of cupric oxide. They are divided into mucins, mucoids, and chondroproteids.

Mucins are secreted by mucous glands and certain mucous membranes. Mucin also occurs in connective tissue and in the

22 to 3 tubes of albumen solution was added respectively (a) crystal of $CuSO_4$ b) (a crystal) $AgNO_3$ c) a small amount of $HgCl$. In each of the three tubes metallic albuminates will be precipitated

umbilical cord. Mucin gives the proteid color reactions, and forms a mucilaginous solution with water containing a little alkali. This solution is not coagulated by heat, but forms a precipitate with acetic acid insoluble in an excess of acid.

Mucoids include colloid and ovamucoid. They occur in the organism and differ from mucins in physical properties and solubility, and are not precipitated by acetic acid.

Chondroproteids yield on hydrolysis chondroitin, sulphuric acid, and an ethereal sulphuric acid in combination with a carbohydrate. This acid and nucleic acid have the power of forming with proteids a compound precipitated by acetic acid, which is occasionally found in the urine, and is called nucleo-albumin. Important chondroproteids are chondromucoid, found in cartilage, and amyloid, found in various organs pathologically.

28. Nucleoproteids, on hydrolysis, yield nucleins. Three varieties are known, differing in hydrolytic products.

(1). Cell-Nucleins yield a proteid, ortho-phosphoric acid, and xanthin bases, and occur chiefly in the nuclei of cells, but also in the protoplasm, and may pass into the animal fluids when the cell is destroyed.

(2). Pseudonucleins yield proteid and ortho-phosphoric acid, and occur in almost all animals and vegetables. Casein of milk is a nucleoproteid containing a pseudonuclein.

(3). Nucleic acid yields ortho-phosphoric acid and xanthin bases, and occurs in the nuclei of the spermatozoa alone.

All give the proteid color reactions, are soluble in water containing a little alkali, and are precipitated from this solution by acetic acid. Nucleins are not decomposed by gastric juice, and are obtained as an insoluble residue after the artificial digestion of nucleoproteids with pepsin.

29. **Albuminoids.** Albuminoids are a group of proteins whose general properties suggest them to be anomalous simple proteids. They consist of a number of bodies which, in their general characters and elementary composition resemble proteids, but differ from them in many respects. They are amorphous. Some of them contain sulphur, and others do not. The decomposition-products resemble the decomposition-products of proteids.

The principal albuminoids are keratin, elastin, collagen, reticulin, and skeletin.

Keratin occurs in the horny portions of the skin and its appendages.

Elastin occurs in connective, especially yellow elastic, tissue.

Collagen includes ossein the chief organic constituent of bone ; chondrigen of cartilage is a collagen mixed with a small quantity of other material. On boiling with water, more readily with very dilute acid, collagens are converted into gelatin.

Gelatin is obtained by the prolonged boiling of connective tissues, for example, tendon, ligaments, bone, as well as from the substance collagen. Gelatin is a colorless or straw-colored solid, usually occurring in flakes or sheets, swells with water, and when heated dissolves, forming a clear solution, with the property of preventing the formation of precipitates by holding them in suspension in a finely divided condition ; so that they pass through filter paper.

30. Make a watery solution of gelatin (5%) by allowing it first to swell up in the cold water, and then dissolving it with the aid of heat. It is insoluble, but swells up in about six times its volume of cold water.

31. After dissolving with the aid of heat, allow a small portion to cool ; it gelatinizes.

32. Apply the xanthoproteic test for proteids to some of the dissolved portion ; make notes of any differences as compared with proteids in this and in the following tests :

33. Use Millon's reagent.

34. Try Piowtrowski's reaction.

35. Is it precipitated by the acetic acid and potassium ferrocyanide test ?

36. Does it coagulate by heat ?

37. Is it precipitated by saturation with magnesium sulphate ?

38. What is the result of the addition of tannic acid ?

39. Add picric acid (saturated solution); if a precipitate appears apply heat and note any change that may occur upon cooling.

32 Nitric acid was added but no
precipitate was formed; the solution turn
yellow on boiling. Ammonia was ad
and it turned orange color.
33 White precipitate was formed which on
boiling turns brick red.
34 An excess of KOH was added and a
couple of drops of $CuSO_4$. The white turne
violet blue
35 no
36 no
37 no
                                    was or precipitate

40.   What is the effect of adding alcohol to the gelatin solution ?

41.   Add a little solution of mercuric chloride to the gelatin solution.   Note which tests serve to differentiate gelatin from albumin.

42.   Bone.   Organic basis obtained by decalcification. Place a small thin dry bone in dilute hydrochloric acid (1 part of the acid to 8 of water) for a few days.   Its mineral matter is dissolved out, and the bone, although retaining its original form, loses its rigidity, and becomes pliable, and so soft as to be capable of being cut with a knife.   What remains is the organic matrix of ossein.   The experiment in the succeeding paragraph may be carried out in a subsequent exercise after the bone has become thoroughly softened.

43.   Wash the decalcified bone thoroughly with water, in which it is insoluble, place it in a solution of sodium carbonate and wash again.   Boil it in water, and from it gelatin will be obtained.   Neutralize it with sodium carbonate.   The solution gelatinizes.   Test the solution for gelatin.   (31–39).

III

CARBOHYDRATES

44.   The term *carbohydrates* includes an important group of substances, occurring especially in plants.   Starch and sugar make up a large proportion of the parts of plants, while cellulose forms the chief material from which many parts of plants are constructed.   Carbohydrates also occur to a less extent in animals, where they are represented chiefly by glycogen and some forms of sugars.

In elementary composition they are non-nitrogenous and the majority consist of CH and O with the H and O in the same proportion as in water, that is, 2 atoms of H to 1 atom of O.   (This proportion is also obtained in other substances not belonging to the carbohydrate group).

Carbohydrates are indifferent bodies with a neutral reaction and form only loose combinations with other bodies, especially with bases.

————————iling dissolves out. When
solution cools the precipitate reappears
a more granular form.

a precipitate is formed.

mercuric chloride was added to
calcite solution no precipitate was formed

Xanthoproteic test was applied to gelatin but
precipitate was found

The reddish color is not so prominent
with protein.

34 Violet is darker with gelatin.

35 no precipitate on adding acetic acid

36 Albumins do coagulate on heating while
gelatin does not

mercuric chloride did not precipitate gelatin
slight precipitated albumin.

directions were followed
and glue gelatin was formed
the solution gelatinizes
for gelatin Xanthoprotein test ? They say positive result
Reagent Positive Yellow reddish color

Carbohydrates are classified as Monosaccharids or glucoses, (simple sugars) ; disaccharids or saccharoses ; polysaccharids or amyloses.

The monosaccharids $(C_6H_{12}O_6)$ include dextrose (glucose or grape sugar), galactose, levulose, glycuronic acid. They cannot be broken down into simpler sugars.

The disaccharids $(C_{12}H_{22}O_{11})$ on taking up one molecule of water split and yield two simple sugars. Examples are saccharose (cane sugar), maltose (malt sugar), lactose (milk sugar).

The polysaccharids $(C_6H_{10}O_5)_n$ do not resemble sugars. They have no sweet taste, and form simple sugars only after several reactions. Examples are starch, dextrin, animal gum, glycogen, and cellulose.

45. Starch $(C_6H_{10}O_5)_n$ is one of the most widely distributed substances in plants, and it may occur in all the organs of plants, either (a) as a direct or indirect product of the assimilation of CO_2 in the leaves of the plant, or (b) as reserve material in the roots, seeds or shoots for the later periods of generation or vegetation.

46. Squeeze some dry starch powder between the thumb and forefinger, and note the peculiar crepitation sound and feeling.

47. Place 1 gram of starch in a mortar, rub it up with a little cold water, and then add 50 cc. of boiling water. Transfer to an evaporating dish and heat for ten minutes over boiling water. Does the starch go into solution? Filter and test with a drop or two of the iodine solution.

48. Add powdered dry starch to cold water. Is it insoluble ? Filter and test the filtrate with a solution of iodine. A blue color denotes the presence of iodide of starch.

(Prepare the iodine solution as follows: Dissolve 2 grams of potassium iodide in 100 cc. of distilled water, add 1 gram of iodine and shake until dissolved.)

49. To some of the boiled portion of starch, add solution of iodine. Heat and note any change that occurs. If not boiled too long another change may occur when cooled.

50. Render some of the starch mixture alkaline by adding slight excess of caustic potash. Add iodine solution. What is the result ?

One gram of starch was placed into an
evaporating dish with a little cold H_2O and
mixed thoroughly then 50 cc of boiling H_2O was
added and the evaporating dish was placed over
H_2O boiler and allowed to stand for 10 min —
fire. The starch goes into solution. After
ing the solution turned blue when iodine was
added.

Cold H_2O added to pulverized starch does not
cause it to become soluble

To boiled starch was added a solution of Iodine
and heated, It turned colorless after cooling
starch H_2O again became blue as originally

Some of the starch mixture was made
alkaline by adding slight excess of caustic
soda. Iodine solution was added with no effect.
~~Try again, 2~~
a little more I. was added

51. Acidify with dilute sulphuric acid, then add iodine. What is the result?

52. Add some solution of tannic acid. Note result and then heat.

53. Place some strong starch mixture in a dialyzer and the latter in distilled water. Allow it to stand for some time and test the water for starch.

54. Saturate a portion of the starch mixture with crystals of ammonium or magnesium sulphate. Filter. Dilute the filtrate with an equal volume of water and add a drop or two of the iodine solution. Is the starch precipitated by the salt?

55. *Glycogen* $(C_6H_{10}O_5)_n$ is a polysaccharid found exclusively in animals chiefly in the liver, in the leucocytes, in all embryonic tissues, and in muscle. It is also known as animal starch. It forms an opalescent solution in water, gives a red color with iodine. On boiling with acids it is converted into dextrin, then maltose and dextrose. The amylolytic enzymes, by hydrolysis, produce the same changes. Basic lead acetate precipitates glycogen. Barfoed's reagent is not reduced.

56. *Dextrin*. $(C_6H_{10}O_5)$ is an intermediate product in the hydration of starch.

57. Dissolve some dextrin, about 2%, in boiling water (100 cc.) and cool. Add iodine solution—a reddish brown color, which disappears on heating and returns on cooling. (The student should take two test tubes placing the dextrin solution in one, and an equal volume of water in the other. Add to both an equal volume of iodine solution and thus compare the difference in color.) Dextrin is made commercially by heating starch to 200° C.

58. Saturate a solution of dextrin (57) with ammonium sulphate. Note result. Filter. Dilute with an equal volume of water and test the filtrate for dextrin.

59. Test a solution of dextrin (57) with Barfoed's reagent and heat. Barfoed's reagent is made by dissolving 13 grams of cupric acetate in 200 cc. distilled water and add 5 cc. commercial acetic acid (38%).

60. Test a solution of dextrin (57) with a few drops of a solution of *basic* lead acetate. Is there a precipitate? (The lead acetate *must be basic*. To insure this the solution of lead

$+ H_2 SO_4 + 9$

...eavy black color is attained upon ...ting (Try again) Blue result ... Starch solution + tannic acid ...re a yellowish precipitating liquid ...hich upon heating became almost ...rless.

? Some starch solution was put into dialyser this was then put into distilled ...o, after some time the distilled H_2O tested for starch. that was negative.

"Starch is ~~not~~ precipitated by salts ammonium and magnesium ...sulphate. (Try again).

2 Bottles of were taken in one some ...tine was put in the other H_2O to both ...these were added a little Iodine solution the ...this became reddish brown which upon ...ting became colorless this was allowed to cool ...d it regained its first color. The H_2O ...ame ~~red~~ yellow-brown colored also. ex~~cept~~ no ...ange was noted in heating & cooling

acetate may be boiled with litharge for ten minutes, the filtrate will be basic lead acetate.)

61. *Cellulose.* $(C_6H_{10}O_5)_n$ occurs in every tissue of the higher plants, where it forms the walls of cells and the great mass of hard parts of wood. It is also found in the outer investment of the animals known as Tunicates. Purified absorbent cotton and filter paper are good examples of cellulose. Cellulose is insoluble in the ordinary solvents, but can be dissolved in the strong mineral acids, being converted into dextrin. Iodine does not stain the unaltered cellulose, but does so after it has been acted upon by the acid. Cellulose is only slightly attacked by the digestive ferments of man, though the herbivorous animals digest it to a greater extent. By the continued action of acids it is converted into glucose.

62. Immerse a piece of filter paper or absorbent cotton in a 1% solution of potassium iodide. Let dry. Immerse for an instant in sulphuric acid and then immediately rinse in water. If cellulose is present a blue color will appear.

63. Schulze's reagent. Iodine dissolved to saturation in a zinc chloride solution of specific gravity 1.8 to which 6 parts of potassium iodide has been added, will turn cellulose blue.

64. Immerse a strip of filter paper for a moment in concentrated sulphuric acid. Then rinse it immediately in plenty of cold water. If the time of immersion has been correct, the paper will be semi-transparent after washing, and as tough as an animal membrane. It is called vegetable parchment and can be stained blue by iodine.

_____

IV

65. *Dextrose or Glucose* (Grape sugar) $(C_6H_{12}O_6)$ exists in fruits and in small quantities in the blood and other fluids and organs. It is the form of sugar found in diabetic urine. It is readily soluble in water. Use 100 cc. of a 2% solution. Dextrose is made commercially by boiling starch with a dilute acid.

66. To a portion of this solution add a little iodine solution. Compare with starch.

...turned the

...textrin to light blue color.

no change up on heating.

Dextrin + Basic lead acetate = white silky precipitate appearance (almost same (the dextrin) does basic

ad acetate precipitate yes

Test was Positive. dextrin?

Schulze's reagent was acted positive manner. Turned cellulose blue.

Filter paper treated with H_2SO_4 a rinsed in cold H_2O the paper became semi-transparent after washing, it also became tough. It stains blue by Iodine.

Starch Solution turned blue { on adding
Dextrin " " " cherry } Iodine
Solution

67. Heat another portion of the solution with sulphuric acid ;—it darkens slowly. If not successful add more dextrose and repeat.

68. Trommer's test. To another part of the solution add a few drops of a dilute solution of copper sulphate, and afterwards add caustic potash in excess, that is, until the precipitate first formed is re-dissolved and a clear blue fluid is obtained. The hydrated oxide of copper precipitated from the copper sulphate is held in solution in presence of glucose. Heat slowly turning the tube in the flame. A little below the boiling point, if glucose be present, the blue color disappears and a yellow (cuprous hydrate) or red (cuprous oxide) precipitate is obtained. If the upper surface of the fluid has been boiled, the yellow precipitate, when it occurs, contrasts sharply with the deep blue-colored stratum below. The precipitate is first yellow, then yellowish red, and finally red. It is better seen in reflected than transmitted light. If no sugar be present, only a black color may be obtained.

69. Fehling's solution. Solution A. 34.64 grams of pure crystalline copper sulphate are powdered and dissolved in 500 cc. of distilled water. Solution B. Sodio-potassium tartrate (Rochelle Salts) 173 grams. Pure caustic potash 125 grams. Add enough distilled water to make 500 cc. When needed for use take equal parts of solutions A and B.

The above stock solutions have been made and each student is to take 30 cc. of each solution. Keep in separate bottles and mix a few cc. of each when ready to make a test. A deep clear blue fluid is the result of the mixture, the Rochelle salt holding the cupric hydrate in solution. If kept too long it is apt to decompose. If in doubt as to the efficiency of the solution boil it, and if it remains blue it is good.

Add some of Fehling's solution to a portion of the glucose ; boil, a yellowish (cuprous hydrate) or reddish (cuprous oxide) precipitate.

70. Add to a portion of the glucose solution some strong potassium hydrate solution and then a very small amount of the subnitrate of bismuth. Boil ; a black precipitate results which sometimes forms a mirror on the walls of the test-tube. This is known as Boettger's test. Albumin gives the same

[handwritten margin note: First yellow then yellowish red + finally red]

——————————————— — ushore was diluted
the sulphuric acid   and heated it
changed to darker colors as it was heated,
finally became very black.

Dextrose solution + a few drops of
per sulphate solution + caustic potash
a blue precipitate thus + heat give
yellow color which shows that sugar
present. ~~Heat longer and indi-cate res~~

a portion of Fehling's solution
is added to glucose and boiled, a
more precipitate ~~resulted~~. Heating longer we got
↓ a reddish precipitate.
~~eat longer and indicate~~

Glucose + potassium hydrate
solution + a little subnitrate of bismuth
heat gives a black color.

reaction if present must be removed if a reliable sugar
test is to be obtained.

71. The Phenyl-hydrazine Test. To 5 drops of phenyl-
hydrazine and 10 drops of glacial acetic acid in a test tube is
added 1 cc. of a saturated solution of sodium chloride. After
shaking the mixture, add 3 cc. of the dextrose solution and
heat the test tube for about two minutes. The fluid is then
allowed to cool slowly in order that the crystals may form.
The canary yellow precipitate may be examined in from 20 to
60 minutes under the microscope for the characteristic glu-
cosazone crystals.

The following test also gives good results, but is longer : The Phenyl-
hydrazine Test. To about 10 cc. of the glucose solution in a test-tube add 0.2
gram of phenylhydrazine hydrochlorate, and 0.3 gram of sodium or potassium
acetate. Boil in the water-bath for 20-30 minutes ; then cool the test-tube by
allowing cold water to run upon it and set it aside. A yellow crystalline preci-
pitate is formed which is known as phenyl-glucosazone. Examine some of this
precipitate under a low power of the microscope and note the needle-like and
feathery crystals sometimes arranged in the form of rosettes. Phenyl-glucosa-
zone has a melting point of 204°C.

72. Conversion of starch into glucose. Boil some of the
starch solution with a few drops of sulphuric acid until the
fluid becomes clear and a few drops of it give no blue color
with the iodine solution. Neutralize a small portion with
sodium carbonate ; test it for glucose.

73. Crush a piece of condensed yeast about the size of a
pea. Place it in a test tube and add 10 cc. of the dextrose
solution. Agitate thoroughly and transfer the mixture to a
saccharometer. Leave in a warm place for 24 hours. If fer-
mentation occurs bubbles of carbon dioxide will be found in
the long arm of the saccharometer.

74. Test a portion of the dextrose solution with Barfoed's
reagent. Compare with Fehling's.

75. *Lactose.* Milk Sugar, $(C_{12}H_{22}O_{11}+H_2O)$. This is a
reducing sugar and is found in the milk of all mammals and
occasionally, during pregnancy, in the urine. Lactose is less
soluble in water than dextrose and is insoluble in alcohol.
With pure yeast it does not ferment. By the action of certain
other ferments, however it undergoes alcoholic fermentation,
with the production at the same time of lactic acid, forming
the drinks known as " koumiss " when made from mare's milk,

5 drops of phenyl-hydrazine was

ed 10cc of glacial acetic acid in a

tube to 1cc of of sodium chloride

lution after this mixture was thoroughly

ken .3cc of dextrose solution was

led the test tube was now heated

2 min. The fluid was then allowed

cool slowly. Crystals formed yellow
precipitate
color.

negative Test. Tried twice. Fehling's

tion Tried in the third time and

was positive.

a Crushed piece of yeast about the

ze of pea was placed into a test

to this was added 10cc of the

rose. After agitating for some time the

lute was transferred to a saccharometer

return to lab it was shown that

nulation had taken place by the

pearance of $2 cc$ of CO_2 in

arm of saccharometer

Barford's reaction gave following results change to

and ______" when from cow's milk. The ordinary souring of milk ___ to the formation of lactic acid from the lactose by micro organisms. Lactose must be transformed into dextrose before it can be assimilated. If injected into the veins it appears in the urine. Use a 2% solution of lactose.

76. Test a portion of the solution with Barfoed's reagent. Compare with the similar test for dextrose.

77. Heat a portion of the solution carefully with sulphuric acid,—it chars slowly. (See 67).

78. Add to another portion excess of caustic potash and a few drops of copper sulphate solution and heat,—a yellow or red precipitate (like glucose).

79. Test another portion with Fehling's solution,—there is a reduction like glucose, but its reducing power is not so great as glucose. It requires 10 parts of lactose to reduce the amount of Fehling's solution that will be reduced by 7 of glucose.

80. Apply the phenylhydrazine test and compare carefully the form of the crystals with those obtained in the dextrose solution.

81. *Sucrose.* Cane sugar, $(C_{12}H_{22}O_{11})$. Cane sugar is found in plants, not in the animal kingdom. It has no reducing power, but is decomposed by heating with acid into a molecule of dextrose and one of fructose (fruit sugar). Make a 2% solution of cane sugar.

82. A portion of the solution should not reduce Fehling's solution. (Many of the commercial sugars, however, contain sufficient reducing sugar to do this.)

83. Trommer's test. Add excess of caustic potash and a drop of copper sulphate (it gives a clear blue fluid), and heat. With a pure sugar there should be no reduction.

84. Pour strong sulphuric acid on a little dry cane sugar in a test-tube. Add a few drops of water with a pipette, the whole mass is quickly charred.

85. Boil a solution of cane sugar with a little sulphuric acid added. Neutralize the solution with a little sodium carbonate and test for dextrose.

86. Apply Barfoed's, Boettger's and the phenyl-hydrazine tests to portions of the cane sugar solution and note if any reduction occurs.

...gives a yellow tinge precipitate.
While dextrose gave a red precipitate
with on top and bottom

Lactose + H_2SO_4 + heat - chars to
brown color

Lactose + excess of caustic potash +
few drops of coppe sulphate + heat gave
at a white then yellow amber color

Fehling's solution + lactose + heat -
rich red color

The phenylhydrazine test was
applied to some lactose - Upon heating
the substance changed color and
on cooling we had red yellow precipitate
the crystals were not the same as)
dextrose. But larger. They were salt.)
Try again, tried again boiled Result two crystals)
Trommer's test.

Cane sugar + $K(OH)$ + $CuSO_4$ ✻ (= Fehling
solution) + heat no reaction

Dry cane sugar + H_2SO_4 + a little H_2O charred turned
black

cane sugar solution + H_2SO_4 + heat + Fehling's

87. *Maltose.* Malt sugar $(C_{12}H_{22}O_{11}+H_2O)$. The reducing power of maltose is one third less than dextrose. Maltose can be easily transformed into dextrose by acids and ferments, but dextrose cannot be converted into maltose. Maltose must be transformed into dextrose before it can be absorbed into the blood. One molecule of maltose decomposes into two molecules of dextrose. Use a 2% solution of maltose.

88. Apply Barfoed's test to a portion of the maltose solution and compare with dextrose.

89. Apply the phenyl-hydrazine test and compare the crystals with those obtained in the dextrose solution.

90. To other portions of the maltose solution apply Trommer's, Fehling's, and Boettger's tests respectively, and compare with dextrose.

V

91. *Fats.* The fats occur in both plants and animals. They are insoluble in water and have a lower specific gravity. They dissolve in hot alcohol more easily than in cold, and are easily soluble in ether, gasoline, or benzol.

Fats are composed of three elements : carbon, hydrogen, and oxygen. They contain a much smaller percentage of oxygen than the carbohydrates, the hydrogen and oxygen not being in the proportion to form water. When the fats are kept at the temperature of superheated steam or subjected to the pancreatic enzyme—steapsin, they take up water and are split into two compounds : glycerine, on the one hand, and one or more of the fatty acids, on the other. They may be considered, then, as made up of glycerine and a fatty acid less water.

This splitting up of the fat molecule is called saponification. It occurs when fats become rancid. It can also be effected by boiling the fat with a caustic alkali. Here, instead of the free fatty acid being left, it unites with the alkali to form a salt. These metallic salts of a fatty acid are the soaps. The soaps of the alkalies are soluble in water, the potassium compound being hygroscopic and forming soft soap. The sodium compound forms a hard soap.

Boettger's   no reaction tried twice

phenyl-hydrazine got a reaction * crystals

maltose + Barfoid's $\overset{+ heat}{(= }$ — Try again

" . Barfred's test turned dextrose to a
lt blue color

Phenyl-hydrazine test. applied to dextrose solution
~ How does maltazone compare
with lactazone?
Trommer's test applied to maltos gave first
less the flesh red color on dextrose we got
= yellow red color.

hling's solution gave a deep red yellow color
"   "   "   gave a red yellow   "
  dextrose.

etger's test on maltose gave a gray
h color while on dextrose it gave flesh color
      —

  Crystals were sheaped like edges of
~ * Dextrose Lactose were fan
ped crystals.

eutral fats. The neutral fats of the adipose tissue of the body generally consist of a mixture of the neutral fats, stearin, palmitin, and olein, the two former being solid at ordinary temperatures, while olein is fluid, and keeps the other two in solution at the temperature of the body. They are lighter than water : Sp. gr. 0.91–0.94.

93. Try the reaction of a fresh fat, like lard or olive oil, with a piece of litmus paper. It is neutral ; but, if the fat has been standing for some time and has become rancid, it may be slightly acid.

94. Test the solubility of a few drops of olive oil in a test tube of water. It mixes when shaken violently, but soon separates at the top on standing. Add now a few drops of a soap solution and shake again. The liquid becomes milky and the fat does not separate. If the oil is not fresh it may be necessary to add a few drops of sodium carbonate to neutralize the free acid.

95. Take a little lard or olive oil, and observe that fat is soluble in ether, also chloroform. Take some of the ethereal solution of lard and let some of it fall upon some paper. The ether soon evaporates but a permanent greasy stain is left.

96. Shake a few drops of cod-liver oil with a small amount of dilute solution of sodium carbonate. The mass should become white—an emulsion. In an emulsion the particles of oil are broken up into innumerable finer particles which remain discrete, that is, do not run together. Milk is a typical emulsion. Examine some of the cod-liver oil emulsion under the microscope.

97. To about 10 grams (11 cc) of olive oil add 20 cc of a 10% solution of caustic potash. Boil the mixture, gently stirring, meanwhile, until the odor of the oil has largely disappeared and it appears homogeneous and no oil separates when a few drops are poured into water. This may require half an hour. Add water as the solution evaporates, to keep the original volume. The product is a mixture of potassium soap and glycerine.

98. Convert a portion of the above soap into the sodium or hard soap by adding some saturated salt solution and allowing it to stand until cold. It will dissolve on warming.

Olive oil did not change the color of the litmus paper ∴ it must be neutral.

Olive oil + little H_2O in test tube did not mix when thoroughly shaken the oil raises to top. This + a little soap solution and then roughly shaken the liquid becomes milky and the fats do not separate.

Olive oil is soluble in chloroform. Some of the solution was put on paper a grease spot remained.

Olive oil + dilute solution of sodium bornate shaken up gives a white colored emulsion. Under the microscope we see many oil globules

11 cc of Olive oil + 20 cc of 10 % solution caustic potash + heat gave a homogeneous solution. The product is a mixture of Potassium and glycerine.

Above solution + sodium chloride gave sodium

99. To another portion add some solution of calcium chloride. A calcium soap is formed which is insoluble in water. It is this compound which is produced by the action of soap on "hard water." Many of the heavy metals give similar compounds. Solutions of lead, iron, copper, etc., may be tried.

100. To the remainder of the potassium soap solution add sulphuric acid slowly until it is plainly acid to test paper. The fatty acids are set free as insoluble substances, the glycerine remaining in solution. Filter out the acids by means of a wet filter paper, through which the acids will not pass. The filtrate contains the glycerine, and must undergo still further treatment before the glycerine can be obtained in a pure form.

VI

EXAMINATION FOR A TEST SOLUTION OF PROTEIDS AND CARBOHYDRATES

101. Note the physical characters of the solution as to color, transparency, odor, and taste. A persistent froth suggests an albuminous solution. Filter the solution if not clear. Divide it into two portions, and follow the outlines below.

A. Proteids.

Test reaction to determine if acid or alkaline.

Neutralize. If a precipitate forms it is acid or alkali albumin. If either is present, filter.

Pour a few drops of the filtrate into water. A precipitate or turbidity shows globulins, if present.

Pour remainder of filtrate into excess of water and filter.

To this filtrate add acetic acid and sodium sulphate and boil. Albumins, if present, are precipitated. If precipitate is formed, filter.

Test filtrate for gelatin. If present, saturate *thoroughly* with ammonium sulphate (crystals) and filter off precipitated gelatin.

Test filtrate for peptones. Biuret test (cold).

..., so an...ous ...tion was added some ...cium chloride. This when cold did ...t dissolve out but broke up into small ...ticles

The remainding soap solution + H_2SO_4 ... filtered after it had an acid reaction ... filtrate contained the glycerine, while ... residue on filter paper contained the ...ty acids

———————

VI

———————

...utral

..., globulins

...bumins are present. (Solution + acetic acid ... sodium sulphate + heat gave a white precipitate)

... gelatin present (...ksworth picric + tannic acid tests)

...tones are present (Biuret or Pirotrowski's test) ...ve a faded pink color.

B. Carbohydrates.

Test original solution for starch.

Then saturate *thoroughly* with ammonium sulphate (crystals). Starch and glycogen, if present, are precipitated, together with proteids. Dextrin, if present, will remain in solution. Filter, and save precipitate(*a*) and filtrate (*b*).

(*a*) Wash precipitate on filter with small portions of a saturated solution of ammonium sulphate till portions of washings give no *trace* of dextrin. (In testing washings for dextrin dilute each time with an equal volume of water). When washings are entirely free from dextrin pass two or three cc. of water (cold) through filter and test for glycogen with a single drop of iodine. A red brown or mahogany color results if glycogen is present. Basic lead acetate precipitates glycogen but not dextrin.

(*b*) Dilute filtrate (*b*) with an equal volume of water and test for dextrin.

Test original solution for reducing sugars—first precipitating out the proteids with acetic acid and sodium sulphate and boiling.

* * *

VII

SALIVARY DIGESTION

102. The saliva is a mixture of the secretions of the parotid, submaxillary, and sublingual glands with that of the glands of the membrane of the mouth. The reaction of the mixed saliva is usually alkaline but may on fasting, also during the night toward morning, and 2–3 hours after meals, or after much talking, become acid. On standing some hours it may become acid and a film of calcium carbonate form on the surface. The normal mixed saliva contains inorganic constituents consisting of : carbonates, chlorides, sulphates, and nitrites of magnesium, calcium, potassium, and sodium, also the sulphocyanide of potassium. The nitrites and sulphocyanide are often absent. The organic constituents are albumin, mucin, and ptyalin. The ptyalin has the power to convert

3

no starch in solution (added I but it did not
a blue, even heated it no results.

no dextrin present.

Sly cogen is present.

extrose.
actose.
altose.   not Present

starch into dextrin, maltose, and some dextrose. It is not able to penetrate the granule of unboiled starch, or does so very slowly, differing in this respect from the corresponding enzyme of the pancreas. It acts best at about the temperature 40° C. Ptyalin is destroyed by acids—especially the mineral acids. In the saliva of some animals, as the horse, the enzyme is not present in the free state but as a zymogen from which it readily forms in mastication. [Novy].

To obtain mixed saliva. Chew a small piece of paraffin or chewing gum, or inhale ether for a short time to stimulate the flow of the secretion. Collect it in a graduate until you have about 50 cc. Note that, in a short time, more or less of a sediment occurs due to the deposition of epithelial cells, débris of food, bacteria, etc. Numerous air bubbles are usually present upon the surface.

Filter. Is it translucent ? Is there any great amount of viscidity ? What is its reaction to litmus paper ? The specific gravity is 1002–1006. Test the specific gravity with an urinometer.

· 103. To a small portion add acetic acid.. A precipitate indicates mucin. Not soluble in excess.

104. With another portion test for traces of proteids with the xanthoproteic reaction and Millon's test.

104½. To a few drops of saliva in a porcelain evaporating dish add a few drops of dilute acidulated ferric chloride,—a red coloration indicates the presence of sulphocyanide of potassium, the color does not disappear on heating, nor on the addition of an acid, but is discharged by mercuric chloride. Meconic acid gives a similar color, but it is not discharged by mercuric chloride. The sulphocyanide is present only in the secretion from the parotid gland.

105. Test for nitrites with a few drops of a starch solution acidified with a little dilute sulphuric acid and containing a small amount of potassium iodide. A nitrite immediately gives a blue color.

106. Test for chlorides by adding to the saliva a few drops of nitric acid followed by a few drops of silver nitrate. A white precipitate indicates the combination of the chloride with the silver to form silver chloride.

Salivary digestion

Saliva is translucent. neutral,
acid (little) S.G. 1.0025

3 Saliva + acetic acid showed a
precipitate which indicates mucin
present.

1 Proteid present with xanthoproteic
action and Millon's test.

½
Sulphocyanide of potassium is present

Slight indication of nitrites.
Test for chlorides Positive.

107. Test another portion of the saliva with a few drops of barium chloride for sulphates.

108. Digestive action on starch. Prepare a mixture by placing 1 gram of starch in a mortar and adding a few cc. of cold water, and mix well with the starch. Add 200 cc. of boiling water, stirring all the while. Boil the fluid for a few minutes. This gives a 0.5% mixture.

109. Dilute the saliva with an equal volume of distilled water. Label four test tubes, A, B, C, and D. Into A place some saliva, boil it and later add some starch mucilage. In B and C, place starch mucilage and saliva, to B add a few drops of hydrochloric acid and to C some caustic potash. To D add merely the saliva to the starch mixture.

Place all four in a water bath not exceeding 40° C., and after a time test a small portion of them for sugar with Fehling's solution. Reserve a small amount of D. Why is no sugar formed in A ? In B and C a strong acid and alkali arrest the action of ptyalin. Neutralize a portion of B and C and test again. Is there any result ? In D the starch has been converted by the ptyalin into a reducing sugar.

110. Test portions of D with Fehling's and iodine solutions. The absence of any blue color with the iodine indicates that the starch has disappeared, having been converted into a reducing sugar—maltose. Also test the remainder of A, B, and C with the iodine solution.

111. Test another portion of D with phenyl-hydrazin, (71) crystals of phenyl-maltosazone should develop. Examine under the microscope.

112. The intermediate products of salivary digestion may be detected by proceeding as in D (109) and testing a few drops of the mixture every two minutes with a drop of iodine upon a porcelain plate. At first there is a blue color denoting *soluble starch*; later there is a reddish violet color indicating the presence of *erythrodextrin*; still later there is only a slight yellowish brown color, or no color at all, when the drop of iodine is added, and this indicates *achroodextrin*—the achromic point—when a reducing sugar maltose is also present. At this point the solution should reduce Fehlings. Any undigested starch may be precipitated by alcohol which leaves the maltose in solution. Saturation with ammonium sulphate

17 Test for sulphates Positive

9 A. No sugar in A   Boiling spoiled
   B. A little sugar   B no sugar (not
   C. "   ~X~ " ..   2nd C No sugar } even
   D. ~/~ X~ " "   time D Reducing sugar present (neutralizing)
                                (Maltose)

   D + Fehling's solution gave a green yellow
   + color shows sugar, probably maltose
   sent

   D + Iodine no blue color ∴ starch
   ~ disappeared having been converted
   to maltose, a reducing sugar.

   + Iodine = blue color   starch present
   +   "   '   '   '   "   "
   +   "   "   no color. no   '   "

   D + phenyl - hydrazin, soc~ got faint
   vurane of crystals.

   D + (9 min time) H₂O ~~soluble~~ 40°C + little
   gave starch (soluble starch)
   D 9 min later + I gave reddish
   let colr. (erythrodextrin

   ~ ... later D + I gave a yellow~

crystals also precipitates the starch but does not affect the dextrins or maltose.

113. The effect of drugs on salivary action. The following may be used : carbolic acid 2%, saturated aqueous solutions of salicylic, benzoic and boric acids, corrosive sublimate 1—1000, quinine bisulphate 2%, alcohol 50%. Place in each test tube 2 cc. boiled starch 2%, 2 cc. sodium carbonate 5%, 1 cc. saliva, 5 cc. of the given drug. Shake and set in the water bath at 40° C for an hour. The activity of digestion may be compared by testing a small portion of each tube with the iodine solution, to see if the starch has disappeared ; or another small portion with Fehling's to see if maltose has formed. A cruder method is to add to each tube an equal volume of 10% caustic soda or potash with a little dilute copper sulphate solution (Trommer's test). The amount of precipitate or depth of color roughly corresponds to the amount of digestion.

114. Bread. Crumble up a small piece of bread in a test-tube and add some cold distilled water until it softens and with slight shaking disintegrates. Divide the mixture into two portions.

115. Apply a drop of iodine solution : blue color indicates starch.

116. Apply the xanthoproteic reaction to the other portion. Any crumbs that may be in the solution if colored orange would indicate the presence of a proteid. The liquid portion may not show as deep a color, indicating a lesser amount in solution.

117. Try the above tests hurriedly by dropping a little iodine solution upon the bread. Similarly with the xanthoproteic test by letting a drop of nitric acid fall upon the bread and then a drop of ammonia upon the spot already covered by the nitric acid.

118. Potato. Boil a small piece of potato in water and let it cool. Divide the liquid into two parts.

Test one portion for starch with the iodine solution. Without boiling, the starch might give no reaction as the granules are enclosed in a coating of cellulose.

119. Apply the xanthoproteic test. Only a faint orange color appears, indicating that very little proteid is present.

... present.

3.
...lic acid = no starch   Sugar present
...ic " = A little "   " "
...ic acid " no "   " "
...ic " " " "
...sive sublimate = Starch present   " "
...mine bisulphate = "   " "
...cohol = no " "   " "

5   Iodine is present.

.   Sorted is present

  Starch is present

6   Proteid is present.

7   Starch is present

9   A slight amt of protein is
...sent.

VIII

Begin

GASTRIC DIGESTION

120.   The gastric juice, secreted by the glands of the
stomach, differs from the other digestive fluids in having an
acid reaction.   It is a clear thin liquid having a specific
gravity of 1002–1006.   The average composition of man's gas-
tric juice is as follows :

Water	99.26
Pepsin rennin and other organic matter	0.30
Free hydrochloric acid	0.22
Alkali chlorides	0.20
Phosphates of alkalies,	
calcium, magnesium and iron	0.02

There is more hydrochloric acid than can unite with the
bases, and this must consequently be in the free state.   The
most important of the organic substances are the two enzymes:
pepsin and rennin.   Differences exist in different animals, *e. g.*,
in carnivora there is a higher percentage of acid than in
others.

An artificial digestive fluid giving very good results may
be made by dissolving 0.3 gram of commercial pepsin in 1000
cc. of a 0.2% solution of hydrochloric acid.

It is more desirable in many ways, however, to prepare an
extract from the gastric mucous membrane itself.   The writer
has found the following method to answer very satisfactorily.
To each gram of the mucous membrane add 1 cc. of a 1%
solution of acetic acid.   Triturate thoroughly in the mortar;
then add 10 cc. of chloroform water for each gram of the
mucous membrane.   This may be kept for some time.   When
ready to use, filter and add 2 cc. of the extract to 8 cc. of the
0.2% hydrochloric acid, or equal volumes of the extract and
the acid may be used.

From a comparative stand point, extracts may be made
from each of the three great groups of animals : Omnivora
(pig), carnivora (dog), and herbivora (horse or cow), and
differences in the rate of digestion noted.

121.   Label 6 test tubes, A, B, C, D, E, F.   In A fill the
tube half full of distilled water, and add 30 drops, or 2 cc. of

	Fibrine		
Swollen & slightly broken up		neg	Pos
3. Swollen a little		neg.	Pos
slightly swollen & broken up		Pos	Pos
swollen a little		neg	Pos
Swollen & Broken up		Pos	Pos
Very much broken up		neg	Pos
Slightly swollen		neg.	neg.
Swollen slightly		neg	Slightly
Swollen & broken up		Pos	Pos
Swollen very little		Slightly	Pos
Swollen very much		neg	Pos
Swollen little coloured green		Pos	Pos

the extract. Fill B half full of 0.2% hydrochloric acid. Treat C similarly to B, but add 30 drops of the extract. Fill D half full of a 1% solution of sodium carbonate and add 30 drops of the extract. Place 30 drops of the extract in E and add 2 or 3 cc. of distilled water and boil, then add enough 0.2% hydrochloric acid to make the tube half full. In F put 2 cc. of the extract and 2 cc. of bile and fill the tube half full of 0.2% hydrochloric acid. In each of the 6 test tubes put a small thread of well-washed and boiled fibrin. Place all the tubes in a water bath at 40° C., and after an hour note any changes that may have occurred in any of the tubes. The rapidity of action will indicate the strength of the ferment. Explain in your notes why no action has occurred in certain of the tubes. Test-tube C is to be plugged with cotton and reserved for later examination, in the next exercise.

122. The following tubes are to be prepared exactly as C but omitting the fibrin. In the first tube a very small piece of meat ; in the second a crumb of bread; in the third a bit of boiled potato ; in the fourth a small piece of dried albumin ; in the fifth add a small piece of butter ; in the sixth, 1 cc. of milk diluted with 5 cc. of distilled water ; in the seventh test-tube a small piece of gelatin ; in the eighth tube small amounts of all the above substances. These tubes, also, are to remain in the water bath at 40° C., and tested later for intermediate and end products. (See 124, 125, 127).

IX

123. The contents of tube C is to be divided into 4 parts, 3 of which are to be used in the following tests, and the other part to be held in reserve, if needed, to correct any of the other tests.

124. Color one portion of the fluid with the litmus solution and neutralize by the contact method with 1% sodium carbonate. (See plate I). At the neutral zone a precipitate will appear indicating acid-albumin (syntonin). The contact test without the litmus is equally delicate.

125. Add to another portion of the solution enough crystals of neutral ammonium sulphate to saturate it. This brings

	Acid Albumin	Albumen	pept.
Horse	pos.	pos.	pos.
Dog	"	pos	pos
Horse	neg	pos	pos
Dog	no	pos	pos
Horse	neg	pos	"
Dog	neg	pos	"
Horse	neg		
Dog	neg	neg	pos
Horse	neg	"	slight
Dog	neg	pos	positive
Horse	pos	"	
dog	pos		
Horse	neg	"	"
Dog	"	"	"
Horse	pos	"	"
Dog	"	½	"

1. Red color.

Neutralized and then was a white
ipitate between red & blue color
id albumin - positive.

5. C + Ammonium sulphate.
biuret test gives albumose
sent

down the albumoses or proteoses in the form of a white precipitate. Albumose like peptone is soluble in water, and gives the biuret reaction. Ammonium sulphate precipitates all of the proteids but peptone.

Another test for albumose is to add sodium chloride and a few drops of nitric acid. A precipitate should appear which is dissolved on heating, but reappears on cooling, indicating the presence of albumose.

126. Peptones behave differently from the native proteids in the copper sulphate and caustic potash test, if only a trace of copper sulphate is used. They give a pink instead of a violet color. (Also true of albumoses). The pink color is also given by the substance called biuret, hence the test is often called the *biuret reaction*. (Biuret is formed by heating urea ; ammonia passes off and leaves biuret, thus : $2CON_2H_4$ (urea)—NH_3 (ammonia) equals $C_2O_2N_3H_5$ (biuret).

127. To the third portion add neutral ammonium sulphate to saturation. This precipitates all of the albumoses and proteids while the peptones remain in solution. Filter and test the filtrate for peptones by the biuret test as follows: Take another test-tube and put a few drops of 1% solution of copper sulphate in it ; empty it out so that the merest trace of the copper sulphate is adherent to the wall of the tube, then add the filtrate and a few drops of strong caustic potash. A pink color (biuret reaction) should be produced.

128. If digestion has been quite long and complete the tests for acid-albumin and albumose may not be very satisfactory as these substances may have been converted into peptones. They are more readily found shortly after digestion has begun. The main fact, however, that an indiffusible proteid, before being converted into a diffusible peptone, must pass through intermediate forms—acid albumin and albumose—is important, and must be kept in mind in this and succeeding experiments.

129. After filtering, treat the contents of the tubes containing meat, bread, potato, albumin, butter, milk, gelatin, and mixed substances according to the above tests.

130. Drugs on gastric digestion. Use the same preparations as in 113. Put in each test tube 4 cc. 0.2% hydrochloric acid, 2 cc. gastric extract and 4 cc. of the given drug. Keep the tubes at 40° C. for a number of hours or over night and test for peptones.

2.   c was filled & tested and
...eptones were found to be present.

0, Carbolic acid =   home   drug
2 Saligic   =   nyt   no
   Benzoic   =   4   Oept
   Borbi   =   o   "
   Common Sublimate =   "   "
   Bisulphate   =   no   "
   alcohol   =   Oept   "
                           "   c

131. Take two pieces of moist fibrin of equal size. Tie one of the pieces in a bunch with thread and place it in a test tube containing some gastric extract and 0.2% hydrochloric acid. Tear the other piece of fibrin into small flakes and place it in another test tube with the same amount of extract and acid. Let the two tubes digest at 40°C. for an equal length of time and note in which most digestion has occurred. In a crude way this experiment shows the effect of mastication upon gastric digestion. Large lumps are acted upon slowly and with difficulty, while an equal amount of material in a state of fine division is readily digested.

132. *Rennin* or chymosin is the milk-curdling enzyme of the stomach. It is apparently a constant constituent of the gastric juice of vertebrates. It is especially abundant in the mucous membrane of the stomach of the calf (rennet).

A solution for experimental purposes may be prepared as in 120. Both rennin and pepsin go into solution. The preparation should not stand too long (2 or 3 days), and should be neutralized with 1% sodium carbonate before using. Pepsin digests rennin in an acid medicine. Commercial rennin may be used in fluid or tablet form experimentally.

133. To 10 cc. of milk in a test tube add a few drops of the fluid extract of rennin or a 1 grain tablet of rennin and keep the tube for some minutes at a temperature of about 38°C. After a short time the milk becomes solid, forming a curd, and after a time the curd of casein contracts and squeezes out a fluid—the whey.

134. Repeat the experiment but first boil the rennin. Compare and explain the result.

135. Half fill a test tube with 0.2% hydrochloric acid. Put in a little fibrin and add a tablet of rennin. Keep at a temperature of 38°—40°C. for a few hours and test for peptones and intermediate products.

136. To 10 cc. of milk in a test tube add a few flakes of commercial pepsin. Keep at a temperature of 38°C. and note if there is any coagulation of the milk. See that the reaction is neutral. Test also for peptones.

ted as stated on page 40 Smaller lumps
ed quicker than larger ones.

3 Result obtained was same as
cribed on Page 40 - 153.

4 It does not curdle when boiled
   enzyme is distroyed.

5 Pept, present also albumiones
humm.

   Milk curdles
   Pept are present.

PANCREATIC DIGESTION

137. The pancreatic secretion is a clear thick alkaline fluid, rich in solids, and possesses very active enzyme properties. It contains at least three distinct enzymes, besides albumin, leucin, fats, soaps and salts. These solid constituents make up about 10% of the secretion. The enzymes occur in the gland in the form of inactive zymogens, but are changed to the active form a few hours after death or by the action of water or acids. The reaction of the juice is alkaline from the presence of sodium carbonate. The extract made from the gland by means of warm water may be acid in reaction from the presence of sarco-lactic acid, especially if the gland is extracted some time after death.

The ingestion of food stimulates the flow of the pancreatic fluid. There is, therefore, no secretion during starvation and it is intermittent in carnivorous animals where some time elapses between meals. On the other hand secretion is going on almost continually in herbivorous animals because digestion is uninterruptedly taking place.

The enzymes found in the pancreatic juice are : trypsin which digests proteids in an alkaline medium, amylopsin which digests starch similarly to ptyalin, steapsin which splits up fats into glycerine and fatty acids, and finally there is some evidence of a milk-curdling enzyme, although the latter is not universally accepted.

A pancreatic extract for digestive purposes with trypsin and amylopsin may be made by running the gland through a food chopper, or triturating it to a pulp in a mortar and adding 1 cc. of 1% acetic acid for each gram of the pancreas, to liberate the enzyme from the zymogen granules. Then add 10 cc. of chloroform water for each gram of the pancreas to extract the enzymes and at the same time, on account of its antiseptic properties, to prevent putrefaction. For use, 2 cc. of this extract may be added to 8 cc. of 1% sodium carbonate, or equal volumes of the two may be employed.

Commercial pancreatin 5 grams dissolved in 200 cc. of 1% sodium carbonate will also serve for experimental purposes.

138. Prepare eight test-tubes. Each test-tube is to be half filled with 1% sodium carbonate and 2 cc. of the pancreatic extract added. To tubes prepared as above add the following : 1, a bit of fibrin ; 2, a small piece of dried albumin ; 3, a piece of meat ; 4, a crumb of bread ; 5, a bit of cooked potato ; 6, a bit-of cheese ; 7, a small piece of gelatin ; 8, a small amount of each of the above substances. Keep these in the water bath at 40°C. Note particularly any changes that may occur in No. 1, and compare with the fibrin digested with the gastric juice. In those tubes which first show signs of digestive action, test the contents for alkali-albumin by neutralization. (Similar to the acid-albumin test the only difference being the reaction of the digestive fluid). Test also for albumoses. Place the tubes in the incubator until the next exercise and, after filtering, again test them for alkali-albumin, albumose, and peptone, as with preceding tests. (124-127). Reserve a portion of the contents of tubes No. 1 and 2 for indol test later. (150).

139. Place a bit of fibrin in two tubes. Half fill the tubes with 1% sodium carbonate. To one tube add 2 cc. of the pancreatic extract. To the other tube add 2 cc. of bile and 2 cc. of the pancreatic extract. Let the tubes digest at 40°C. and note in which tube peptone first appears.

140. Take three test tubes, add 5 cc. of boiled starch mixture and 5 cc. of 1% sodium carbonate to each tube. To No. 1, add 2 cc. of the pancreatic extract. To No. 2, add 2 cc. of bile. To No. 3, add 2 cc. of bile and 2 cc. of the pancreatic extract. Place the three test tubes in the water bath at 40°C. Test a few drops from each tube every minute upon a white plate with a drop or two of iodine and note in which tube the starch first disappears.

141. Prepare two tubes for starch digestion as follows : In one place 5 cc. of 2% boiled starch and 5 cc. of 1% sodium carbonate and 2 cc. of pancreatic extract. In the other place 1 gram of unboiled starch triturated in 5 cc. of cold water, add 5 cc. of 1% sodium carbonate and 2 cc. of pancreatic extract. Place both tubes in the water bath at 40°C. and test at intervals as in No. 140. Continue the digestion long enough to find sugar in both tubes by the Fehling's test.

	all alk.	Peptone	albumose
1 considerable	neg	Positive	neg
2			
3			
4			
5			
6			
7			
8			

Peptones appear in 7 Tube with pancreatic juice alone, pink color

Starch disappears first in 3rd tube or where bile and pancreatic ext. are to-gether.

Starch disappears 1st in No 1, one with boiled starch.

142. Prepare four test tubes as follows, labeling them in order 1, 2, 3, 4, and adding a bit of fibrin to each tube. To No. 1 add 30 drops of the pancreatic extract from the pig, and some distilled water ; to No. 2, the same amount of the pig's pancreatic extract and excess of 0.2% hydrochloric acid ; to No. 3, some 1% sodium carbonate alone ; for No. 4, put 30 drops of the pancreatic extract into a separate test tube, add a little of the 1% sodium carbonate and boil, and then add the fibrin. Put all of the test tubes in the water-bath at 40°C. After a few hours examine them and explain the result.

143. Emulsion Experiment. Shake up a few drops of olive oil in a test-tube with 2 cc. artificial pancreatic juice and 2 cc. 1% sodium carbonate. Place the mixture for a few minutes in the bath at 40°C., and shake again, compare the results before and after warming. If the oil is neutral, there may be no emulsion or only a poor one. The addition of a few drops of oleic acid will improve it. Why ?

144. In another tube with a little olive oil add 2 cc. bile and 2 cc. 1% sodium carbonate, shake and place in water-bath at 40°C. and compare the emulsive effect with 143. Note whether the oil is neutral or not.

145. Action on Fat. For this experiment it is necessary that the fat should be perfectly neutral. Commercial oils usually contain free fatty acids.

The following method has been recommended for neutralization by Kruken-berg : Place the oil in a porcelain capsule and mix it with not too much baryta solution, (baryta mixture is prepared by mixing one volume of a solution of barium nitrate and two volumes of barium hydrate, both saturated in the cold), and boil for some time. Allow it to cool. The unsaponified oil is extracted with ether. The ethereal extract is separated from the insoluble portion and the ether evaporated over warm water. (The flame *must not* be brought near the ether. Let the water come to a boil, put out the flame and then put the dish containing the ether upon the hot water). The oil should now be neutralized.

The cream from milk is usually of a neutral reaction and serves very well in the following experiments.

146. Take two test tubes and place in each 2 cc. of cream —neutral fat. Add 1 cc. blue litmus to color. In the first tube place a small piece of fresh pancreas. Put both tubes in the water bath and observe at intervals. Note if any

1 ~~rather complete~~

2 Best "

3 . Good " " not as much as '

, Good " But not as much as no
3.

3 Two drops of ~~some~~ oil + 2 cc of
~~creatic~~ juice and 2 cc of sodium
tonate put in 40°C H_2O bath helped
emulsification, before doing this it separated
le after it seemed to be emulsified This +
ie acid gave a good emulsion Because
breaks droplets

We get a better emulsi'i cation with
these ie 1 & 3.

unit
Used cream

= The ~~tube~~ containing the Pancreas
it its ~~litmus~~ color and changed
serum color the milk appearance
ing gone

44

change of color occurs in the one with the pancreas, due to the formation of fatty acids by the enzyme steapsin. A fresh watery extract of the pancreas also acts favorably.

147. Another form of the experiment is to mix the oil with finely divided perfectly fresh pancreas in a mortar, and keep it for a time at 40°C. It soon becomes acid, owing to the formation of fatty acids. Test with litmus paper.

148. Action on milk. Dilute 2 cc. of cow's milk with 10 cc. of distilled water in a test tube and add 5–6 drops of pancreatic extract. Keep at 40°C. from ½ to 1 hour. Note any change that has occurred.

149. Divide the above into two parts. To one part add a little dilute acetic acid ; if there is no precipitate it indicates that the caseinogen has been converted into peptones. To the other part apply the biuret reaction for peptones.

150. With the reserved portion from the albumin and fibrin tubes, (138), indol may be found if digestion has continued long enough and if an offensive odor be present. To some of the suspected fluid add 1 cc. of 0.01% solution of sodium or potassium nitrite (fresh) and then a few drops of concentrated sulphuric acid. A pink color indicates the presence of indol.

151. Food test. Use the two digestive extracts furnished. Try the activity of each upon some fibrin and starch solution in an acid (0.2% HCl) medium, and also in an alkaline (1% Na_2Co_8) medium at a temperature of 38 to 40 deg. C. Determine in which medium the best results are obtained and identify the extract.

Test with the extracts the foodstuff provided. Determine if there is proteolytic digestion, by testing for acid or alkali albumin, albumose, peptone and indol.

Determine if there is amylolytic digestion by testing for dextrin and dextrose.

In addition to the extracts furnished for the experiments, collect your own saliva and try its amylolytic action upon the foodstuff.

152. Valuation of Commercial Pepsin. Dissolve 67 mg. of the pepsin in 100 cc. of 0.2% hydrochloric acid.

Add 5 cc. of the above solution to 95 cc. of 0.2% hydrochloric acid. Place 10cc of this diluted pepsin sol. in 1 test tube, 15 cc. in a 2nd, 20 cc. in a 3rd, 25 cc. in a 4th, 30 cc. in a 5th tube. Place all in a water bath at 38-40 degrees C.

Place an egg in boiling water and boil for 15 minutes, then place it in cold water. When cold, wipe it dry, remove the coagulated albumin and rub it through a No. 30 sieve.

Place 1 gram of this disintegrated albumin in each of the above test tubes at 38-40 degrees and shake well, to thoroughly mix, being careful not to lose any of the solution or albumin. Keep at 38-40 degrees for six hours, gently shaking the tubes every 15 minutes.

8. 2 cc of milk + 10 c c of distilled H_2
drop of fresh milk + 40 cc H_2O in tube
we no change

9. no precipitate with acetic acid show
serinogen has been converted into
stones

another fast biuret reaction for
proteins was tried with pink color result
Test was positive.

no middle could be found

Note the time at which digestion is completed in each tube. If the pepsin is of U. S. P. strength, this should be at the end of six hours in tube 1, only a few thin, insoluble flakes at most being left. From results obtained, determine the relative strength of the pepsin. How many times its own weight of freshly coagulated albumin will it digest in six hours ?

153. Valuation of Commercial Pancreatin. Dissolve .28 gm. pancreatin in 100cc tepid water and add 1.5 gm. sodium bicarbonate.

Place 5 cc. of the above solution in one test tube and 10 cc. in a second. Heat some fresh cow's milk on a water bath, to 38-40 degrees, and add 20 cc. of this milk to each test tube. Keep at 38-40 degrees for 30 minutes or longer. Test small portions of each from time to time with Heller's cold nitric acid test. At the end of 30 minutes, no coagulum should be produced in tube one, if the pancreatin is of standard strength. Note the time at which no coagulum is produced in each tube. Repeat the experiment if necessary, using a larger or smaller amount of pancreatin solution as indicated by above tests to digest 20 cc. milk in 30 minutes. From amount of pancreatin solution necessary for this, calculate its relative value.

Using a 1% starch solution, determine how many times its weight of starch the pancreatin will digest in 30 minutes.

154. *Succus Entericus.—Invertase* or (invertin) sugar splitting enzyme. This exists in the succus entericus or intestinal juice and the mucous membrane of the small intestine, which splits up cane-sugar and maltose into dextrose and levulose. A molecule of cane-sugar or maltose takes up a molecule of water, and splits into two molecules—one of dextrose, the other of levulose (fructose.)

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6.$$
Cane Sugar   Dextrose Levulose.
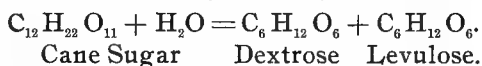
A similar enzyme can be extracted from yeast and many plants.

An extract of invertase may be made from the small intestine by adding to each gram of the mucous membrane used 1 cc. of 1% acetic acid, triturating in the mortar and adding 10 cc. of chloroform for each gram of the mucous membrane. Let the mixture stand for a day or two and before using make slightly alkaline by the addition of 1% sodium carbonate.

155. Place 10 cc. of a 10% solution of cane sugar in a test tube and add 2 cc. of the extract of invertase. Allow the mixture to digest at 40°C. After a short time test with Fehling's solution for reducing sugars.

positive Result was H time

156. Bile is a mixture of the secretion of liver cells and of mucin derived from the cells lining the gall bladder and duct. The bile obtained directly from the liver contains about 2%, and that from the gall bladder contains about 12%, of solids. The difference is due to concentration in the gall bladder and ducts, where also mucinous substances are added. The bile is normally a reddish brown or greenish viscid fluid with a bitter taste and a neutral or slightly alkaline reaction. After a proteid diet the secretion is increased, whereas with fats and carbohydrates it is less marked. The secretion is also decreased in starvation.

The compounds which make up the larger part of the solid matter of the bile are the sodium salts of glycocholic and taurocholic acids. Besides these and the biliary mucin there are present fats, soaps, lecithin, and cholesterin, also a number of inorganic salts of the alkalies, alkaline earths, and iron. The color of the bile is due to the biliary pigments, bilirubin, biliverdin. The source of bilirubin is undoubtedly hematin. On reduction it yields hydrobilirubin which is closely related if not identical with stercobilin (found in the intestines, giving color to feces), and with urobilin of urine. On oxidation bilirubin yields biliverdin. The amount of pigment in the bile is usually only a few hundredths of a per cent., rarely 0.1%. As to the origin of these bile constituents it may be said that the bile acids are elaborated by the cells of the liver, not elsewhere in the body. The bile pigments may possibly be formed in other parts of the body than in the liver, but under normal conditions the liver is the organ where they are formed. Taurin and glycocoll result from the decomposition of proteids in any part of the body. The bile contains no proteids nor formed elements, but in some animals a small amount of diastatic enzyme may be found.

157. Note the peculiar odor of bile. Pour a little from one vessel to another and note the viscidity, due to the presence of mucin and nucleo-proteid.

158. Place some dilute bile (1 to 5) in a test tube and heat to boiling. Immerse a strip of red litmus paper, then remove

Bile

-7 green odor Viscid.

7 Neutral

and wash with water. The reaction should be alkaline if the bile is fresh.

159. To 5 cc. of bile in a test tube add 10 cc. water and then some strong alcohol. This produces a precipitate of mucin with some pigment entangled.

160. Mucin is also precipitated by the addition of acetic acid to bile. Perform this test using the same proportions as in 159. Filter off the mucin.

161. To a portion of the filtrate add a little hydrochloric acid and potassium ferrocyanide. A blue color indicates the presence of iron. The experiment may be modified by placing some thin sections of liver in a solution of potassium ferrocyanide for a few minutes and then in dilute hydrochloric acid. The sections turn bluish from the formation of prussian blue. With the microscope blue granules may be seen in some of the hepatic cells.

162. Test another portion of the filtrates for proteids, also for chlorides and sulphates. Fresh human bile gives no spectrum, but the bile of the ox, mouse and some other animals does.

163. Pettenkofer's Test for Bile Acids. Take 2 cc. of clear diluted bile in a test tube and add 4 drops of a 10% solution of cane sugar. Add strong sulphuric acid, drop by drop, cooling the tube in a dish of cold water immediately after adding the acid. Not more than 2 cc. of the acid should be used. Too much heat causes carbonization of the sugar and the test is ruined. If bile acids are present, the fluid at first becomes opaque, then clear, and successively brown, red and purple. It may require an hour or more to accomplish this test. This reaction depends upon the production of fur-furol (C_4H_3OCHO) by the destruction of the sugar when the sulphuric acid is added. Furfurol in turn combines with cholalic acid, formed by the action of the sulphuric acid on the bile acids, giving the color. Some other substances, as morphine, albumin, etc., give a very similar color, and the test must, therefore, be used with caution. In very dilute solutions of bile the reaction does not appear and cannot be used satisfactorily in testing urine for the presence of bile.

Pettenkofer's test may also be quite satisfactorily per-fomed more quickly by putting a little of the bile in a por-

1 Precipitate is formed.

0 Also got mucin.

1 Positive

2 Positive all required things
ment.

3

Good Positive

celain capsule, adding a drop or 'two of a ·solution. of cane ⤳
sugar and then a few drops of strong sulphuric acid.

164.   Gmelin's Test for Bile Pigments.   Ox gall does not
yield this test as readily as that from the omnivora or carni-
vora.   To a small quantity of bile, in a test-tube, add, drop
by drop, nitric acid, yellow with nitrous acid, (if the acid is
clear, add a single crystal of cane sugar, warm, and the acid
becomes yellow from the development of a small amount of
nitrous acid), shaking after each drop ; the yellowish green
color becomes first a dark green, then blue, then violet, then
red, and finally a dirty yellow.   The blue and violet colors are
less obvious than the rest.

Repeat the test in the following way : place a drop of bile
in a porcelain evaporating dish, and place a drop of yellow
nitric acid so that it runs into the drop of bile ; where the fluids
mingle, zones of color, green, blue, violet, red and yellow, from
the bile to the acid, are seen.

165.   Surface Tension Test.   (Hay).   In a test tube con-
taining some dilute (1–2) bile, sprinkle some powdered sulphur.
Repeat the experiment upon a test tube containing ordinary
water.   Compare the two and note in which the sulphur sinks
the most readily.

166.   Place some diluted bile in a test tube, incline the
tube and add cautiously 2–3 cc. of a dilute tincture of iodine
so that it forms a layer.   In a short time a bright green ring
forms at the zone of contact.   After the ingestion of antipyrin,
the urine will give a similar green ring with iodine.

167.   Acidulate some dilute bile with acetic acid, add a
few cc. of chloroform and shake.   The chloroform dissolves the
bilirubin (not biliverdin) and is colored yellow.

168.   Add a little bile to some starch mucilage as in sali-
vary digestion.   Test for any reducing sugar.

169.   To 5 cc. of undiluted bile add an equal volume of
water and some alcohol. to precipitate the mucin.   Filter and
divide the filtrate into two portions ; to one portion add some
hydrochloric acid which causes a precipitation of glycocholic
acid ; to the other portion add a little of a 1% solution of neu-
lead acetate which throws down lead glycocholate.   Remove
this by filtration, and to the filtrate add a little 1% solution
of *basic* lead acetate, which gives a further precipitation of
lead taurocholate.   [Long.]

Positive worked very fine.

. Sticks first in dilute bile test.

. Positive

Chloroform went to top
dissolved bilirubin. color was yellow

⟶ ?

170. Add a few drops of oleic acid to 5 cc. of bile in a test tube, shake well and at once place a drop of the mixture on a slide and examine, under the microscope, the numerous fatty globules. Place the test tube with the bile in a warm bath for an hour or so, shaking occasionally and then examine a drop with the microscope ; comparatively few fatty globules will be seen. The oleic acid has combined with the base of the bile-salts to form a *soap*.

171. Prepare three test tubes as follows : (1) In one test tube put 5 cc. of bile and a drop of oleic acid. (2) In another 5 cc. of water. (3) In another 5 cc. of bile. To each of the three add about 1 cc, of fresh melted butter or lard. Shake well and place all three in a warm bath. Note in which tube the emulsion continues longest.

172. Free fatty acids have the power of decomposing the bile salts with liberation of their acids. The emulsifying power of bile is slight ; but in the presence of fatty acids it forms soaps, which have a much greater emulsifying power. Animal membranes moistened with bile permit the passage of fatty oils, while if they are moistened with water only the oil cannot pass through. This is important in connection with certain digestive phenomena.

XIII

MILK

173. Newly drawn milk is an opaque fluid of a white color. Its color and opacity are due to its being an emulsion, *i. e.*, consisting of little globules of fat suspended in a solution of albumin, sugar and salts. When the milk is allowed to stand, the fat globules, being lighter than the fluid in which they swim, rise in great part to the top and form cream, and part of the fluid often acquires a bluish tinge. It is said that a similar separation also takes place in the milk gland itself, so that the milk last drawn is richest in cream. The globules of fat are prevented from uniting by the thin albuminous coating (the presence of this coating is denied by some) which surrounds each, but when this is broken by agitation, they

coalesce, forming butter.   Changes also occur in the milk, sugar, casein and fats, more or less quickly, according to the higher or lower temperature to which the milk is exposed. The milk sugar becomes converted, apparently through the agency of a ferment, into lactic acid.   This gives the milk an acid reaction, and precipitates the casein, causing the milk to curdle.   The coagulum or curd, incloses the fat globules. The liquid from which it is separated, a solution of milk sugar. and salts, is known as whey.   The curd, when completely separated from the whey, is called cheese.

The average composition of human and cow's milk and of the cream from cow's milk is given in the following table :

	Human Milk.	Cow's Milk.	Cream.
Water	88.20	86.35	73.35
Proteins	2.00	4.40	4.40
Fat	3.40	4.40	18.00
Lactose	6.00	4.50	4.50
Inorganic Salts	0.35	0.75	0.75

174.   Test the reaction of milk.   Fresh cow's milk may often be neutral or even acid or amphoteric *i. e.*, will color blue litmus red or red litmus blue.   Sour milk is acid.   The reaction of fresh human milk is always alkaline.   Free lactic acid is present in the fresh milk of carnivora.

175.   Determine the specific gravity of unskimmed milk with an accurate lactometer or urinometer.   Allow some milk to stand until the next day.   Remove the cream and again take the specific gravity, then add from 10% to 25% of water and take the specific gravity once more.

The laws of New York require milk to have a density of not less than 1.029, and total solids of not less than 12%, of which 3% must be fats.

Various forms of apparatus for testing milk are on the market.   A convenient apparatus is sold by the Whitall Tatum Co. of New York.   (Apparatus No. 2).   The following suggestions and directions accompany each apparatus :   The lactometer is intended to show the specific gravity or density of milk at the temperature of 60° Fahrenheit.   On the scale **0** represents a specific gravity of 1.000, which is that of water, and **100** represents a specific gravity of 1.029, which has been

5° Br.— (113)

$$\sqrt{\begin{array}{l} 1.029 \\ 00377 \end{array}}$$

.00029
13

1.03277   00087
           00029
           1.00377

established as the lowest specific gravity of the milk from a healthy and ordinarily well-fed cow.

The average lactometer reading of normal milk is 110, and all milk testing below 106 must be regarded as doubtful.

Whenever milk shows less than 106, one of two things may be suspected : *First*, that the milk contains an unusual amount of cream ; *Second*, that the milk has been watered and perhaps skimmed.

It must not be assumed that milk of a low specific gravity is impure, for it may be rich in cream ; nor that milk of a high specific gravity is pure, for it may have been skimmed and its density increased by the removal of the fats. Color, taste and odor will indicate to some extent the quality, but, by the use of the creamometer the percentage of cream in a sample of milk can be determined.

The thermometer is required to determine the temperature of the milk, since the lactometer is correct only when the milk is at 60° Fahrenheit. The following rule for the correction of lactometer readings is sufficiently accurate for ordinary purposes : For each 2½ degrees of temperature *above 60°, add* one to the reading of the lactometer ; example : Lactometer 114, thermometer 70°, or 10° above standard ; add 4 to lactometer reading, making it 118.

For each 2½ degrees *below 60°, subtract* one from the reading of the lactometer ; example : Lactometer 114, thermometer 55°, or 5° below standard ; subtract 2 from the lactometer reading, making it 112.

In testing milk, the following directions should be observed : Fill the creamometer nearly full with the milk ; insert the lactometer and note carefully the point on the stem to which it sinks ; take the temperature of the milk and correct the reading of the lactometer according to the rule given above. If the corrected lactometer reading is less than 106, or if it is suspected that the milk has been skimmed, fill the creamometer to the point marked **0** and place it in a cool place, where it will not be disturbed for twelve hours ; in the summer it can be kept in the refrigerator. At the end of that time the cream will have risen and the percentage can easily be read from the scale on the jar. The lowest safe proportion of cream is 15%, and a percentage lower than that will surely indicate that the milk was poor originally, or has been partly skimmed.

176. Examine a drop of fresh cow's milk under the microscope. It consists of a clear fluid containing a large number of highly retractive fat globules. Let a drop of osmic acid solution run under the cover glass ; in a short time the globules become stained brown-black.

177. To some milk in a test tube add a few drops of sodium or potassium hydrate and heat. The liquid becomes yellow, then orange, and finally brown.

178. To a 4% solution of lactose add some of the same reagent and heat. The same color is developed as in 177, which is due to the sugar present in the milk.

179. To some fresh milk in a test tube add a few drops of fresh tincture of guaiac (20% solution in alcohol); agitate and add some peroxide of hydrogen (or old terpentine). A blue color develops. A similar result is given by the blood.

180. Repeat the experiment with milk that has been boiled. The blue color is not given—due to changes in the proteid.

181. Mix 5 cc. of fresh milk with 15 drops of *neutral* artificial gastric juice, and heat in the water bath to 40° C. In a short time the milk curdles so that the tube can be inverted without the *curd* falling out. By and by the *whey* is squeezed out of the clot. The curdling of milk by the rennin enzyme present in the gastric juice is quite different from that produced by the "souring of milk," or by the precipitation of caseinogen by acids. Here the casein (carrying with it most of the fats) is precipitated in a neutral fluid.

182. To the same test-tube after the above process add 10 cc. of 0.2% hydrochloric acid, and put into the incubator until the next exercise. Note any changes when next examined and test for peptones.

183. Dilute 5 cc. of milk with 15 cc. of water, add a little dilute acetic acid and warm. A precipitate is formed. Filter and save both precipitate and filtrate. This precipitate is not the same as that obtained by rennet. The acid precipitate is caseinogen, and is freely soluble in dilute alkali, the rennet clot is "casein," and is much less soluble in dilute alkali. Cheese is made with rennin and cannot be made with acid.

milk under microscope br, like a mass of globules.

7   Possible   that same a describe

"   Positive

9   ~~zug~~   Positive after 3rd try

0   neg   no blue color.

"   Positive.

-    ~~In incubator~~
On the top was a layer of about ½ in
with non-transparent substance while
the rest of the 1st tube the liquid was ~~but~~

184.   The filtrate obtained from 183 is to be divided into two portions.   To the first portion apply Trommer's test. A red precipitate indicates the presence of a reducing sugar —lactose.

185.   To the second portion of the filtrate apply the xanthoproteic reaction.   An orange color represents the presence of a proteid (lact-albumin).

186.   To the precipitate obtained from 183 add a little ether in a test-tube and agitate for a few minutes.   Pour off the ether upon some paper and note that it leaves a permanent greasy stain indicating the presence of fat.

187.   To the residue left in 186 add a little dilute caustic potash (0.1%).   A solution is effected.   Apply the xanthoproteic reaction to this fluid.   An orange color denotes the presence of a proteid—caseinogen.

188.   To a test-tube half filled with 0.2% hydrochloric acid and 2 cc. of gastric extract add a small piece of cheese. Put the tube in the incubator and examine at the next exercise for peptones and intermediate products.

189.   The action of milk with pancreatic extract is somewhat complicated on account of the complexity of milk itself. The sugar, fat and proteids all undergo some change from the action of the different pancreatic ferments.   Perhaps the most interesting of these changes is that produced in the proteids, and is commonly called peptonization.   The peptonization, or digestion, of milk is quite often practised in the preparation of food for the sick room, and is illustrated by the following experiment.   Dilute about 10 cc. of milk with an equal volume of distilled water and add a half a gram of sodium bicarbonate.   Then add a few drops of pancreatic extract, shake the mixture and keep at 40° C. on the waterbath for about a half an hour.   Then filter and apply the biuret test for peptones.   The pancreatic extract from beef acts more strongly upon the proteids; that from the pig is very active in converting starch into sugar.

190.   Fill a test tube half full of milk and boil it.   Add a tablet of rennin.   Prepare another test tube in the same way, but use fresh unboiled milk.   Place both tubes in the water bath at 38°C.   After some minutes compare the tubes.   The boiled milk should not be coagulated.   The unboiled milk

Reducing sugar

were present.

85 - Protein Good well coln

6 Yes fat is left.

7. Casein & gum present.

5 ~~on incubation~~

Pept was present tested with biuret test

a good pink precipitate.

Peptone were present.

. Boiled milk remained uncoagulated

boiled milk clotted

After a time the whey seperated out

should be clotted. Leave this tube in the water bath if neces-sary, until the *whey* has separated quite completely from the *curd*. Filter and use the filtrate in the following tests.

191. Test one portion of the whey filtrate by adding a few drops of nitric acid and a little ammonium molybdate solution and heat. A yellow precipitate indicates phospates. Test another portion by adding a little silver nitrate. A white precipitate insoluble in nitric acid indicates chlorides (chiefly potassium and sodium). To another portion add a little ammonium oxalate. A precipitate indicates calcium salts. Test the remaining portion for albumin using the xanthoproteic test.

192. To a test tube half full of milk add 3 or 4 drops of a saturated solution of ammonium oxalate ; mix, add a tablet of rennin and digest at 38°–40°C. for at least a half an hour. There should be no coagulum. Then add a few drops of a 2% solution of calcium chloride and digest again. Does the milk coagulate ?

193. Separation of caseinogen by salts. To a test tube half full of milk, add crystals of magnesium sulphate or sodium chloride to saturation. The caseinogen and fat separate out, rise to the surface, and leave a clear salted whey beneath. Caseinogen, like globulins, is precipitated by saturation with $MgSO_4$ or NaCl, but it is not coagulated by heat. It was at · · one time supposed to be an alkali albumin, but the latter is not coagulated by rennin. It appears to be a nucleo-albumin —*i. e.*, a compound of a proteid with nuclein, the latter a body rich in phosphorus.

194. Boil a little milk in a small beaker or evaporating dish. There is no coagulation. A scum forms upon the sur-face which returns as often as it is removed. This is due chiefly to caseinogen entangled in protein drying on exposure to air.

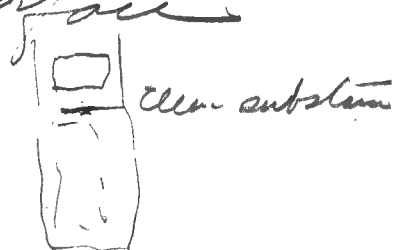195. Place a small quantity of milk in a warm place for one or two days ; then test the reaction, it will be found to be acid ; this is due to *fermentation*, in the process of which the milk sugar is converted into lactic acid.

1) Positive in broth

2 Positive: yes milk coagulated
th itu addition of caclr

3 Searing slained over a day —

9 3 Fat arose to surface
an whey at bottom   clean substan

2 Sum appeard as stated.

XIV

196. An examination of some of the more important tissues of the body.

197. Saline extract of Nervous Tissue. This may be made by triturating the cerebrum with 5% magnesium sulphate in a mortar. After filtering the extract may be used in the following tests. The solids of a saline extract of the brain are derived chiefly from the gray matter, nerve cells.

198. Apply the xanthoproteic test to a portion of the above extract.

199. Faintly acidify another portion with acetic acid and boil. If coagulated a proteid is present. This may be a native albumin or globulin or both.

200. An ethereal extract of the brain or cord contains solids derived chiefly from the white matter (nerve fibers).

201. Pour out a little ethereal extract of brain upon paper and note that there is a permanent greasy stain, indicating the presence of fat. Remember that this is not wholly ordinary fat, but largely a nitrogenous, phosphorated fat— lecithin.

202. To another portion add, *very cautiously*, some strong sulphuric acid. If a cherry red color results at the junction of the fluids it denotes the presence of cholesterin. Make a control test by putting a little ether in a test-tube and adding some strong sulphuric acid. No red color should appear.

203. Saline extract of Liver. (10% NaCl). Divide the fluid into three portions.

204. To one portion apply the xanthoproteic test.

205. Faintly acidify another portion with dilute acetic acid and boil. If coagulated it indicates native albumin or globulin or both.

206. Saturate the third portion with magnesium sulphate. A precipitate should occur of a proteid character. The proteid may be globulin and partly nucleo-albumin.

207. Filter the mixture obtained in 206. Boil the filtrate. Little or no coagulation may occur, indicating little or no albumin in the filtrate.

208. Mince a small piece of liver from an animal which has been dead for 24 hours. Boil the liver either in water or a saturated solution of sodium sulphate. Filter. The filtrate should not be opalescent.

209. Test the reaction of the filtrate to litmus paper.

210. Neutralize a portion of the filtrate with a little sodium carbonate and filter ; then test with iodine for glycogen. If there is no deep brown mahogany color, glycogen is absent.

211. Test for grape sugar by Trommer's or Fehling's tests. After death the glycogen is transformed into grape sugar, unless precautions be taken to prevent this transformation.

212. Saline extract of muscle (10% NaCl). The reaction of perfectly fresh muscle to litmus is of an alkaline character. That of butchers' meat is acid due to the formation of sarcolactic acid. A muscle tetanized for a long time becomes acid.

213. Pour a few drops of the saline extract into a large quantity of water. Observe the milky deposit of myosinogen. The precipitate is redissolved by adding a strong solution of common salt.

214. Test the coagulating point of another portion of the extract. Four proteids are coagulated by heat, each respectively at 47°, 56°, 63°, and 73°C., an albumose being left in solution. The fluid is acid in reaction. Filter off the coagula as they are formed.

215. Saturate the final filtrate with sodium chloride. The myosinogen is precipitated.

216. Collect some of the precipitate of 215 and dissolve it in a weak solution of sodium chloride and test for proteid reactions,—xanthoproteic and Millon's tests.

217. Make a solution of Liebig's extract of meat. Test a small portion of it for proteids.

218. Test another portion for glycogen by adding iodine solution ; a red-brown or port wine color indicates glycogen. Make another test by adding a little basic lead acetate.

219. Test another portion for kreatinin by Weyl's test. Add a very dilute solution of sodium nitro-prusside, and very cautiously some caustic soda ; an evanescent ruby-red color, passing into a straw color, indicates kreatinin.

XV

220. *Blood.* The blood is a red, thick, opaque fluid. The specific gravity varies from 1.045 to 1.075 with an average for adult human beings of about 1.055 ; it depends primarily upon the amount of hemoglobin present.

For examination, it is convenient to consider the blood as composed of two parts : the corpuscles and the albuminous liquid in which they are suspended—the plasma. The solid blood corpuscles in man may constitute nearly one-half the weight of the blood. In some animals as the ox, they make up but one-third of the weight of the blood.

The color of the blood is caused by the red corpuscles. Even comparatively thin layers of the blood are opaque from their presence. The coloring matter (hemoglobin) can be set free from the corpuscles by water or by many chemical reagents. The color then becomes much darker, since the light is no longer reflected from the surface of the corpuscles. The addition of strong neutral salt solutions to blood turns it bright red, because of the increased reflection of light from the shriveled corpuscles.

221. Fresh blood may be obtained and defibrinated at a slaughter house, and a few drops of formalin added· to it will prevent putrefaction for some time. It is better, however, when possible, to obtain the blood by bleeding an animal. After the dog, or any other animal of convenient size, has been anesthetized, the carotid or femoral artery is exposed and isolated from surrounding parts for an inch or two of its length, and a clamp or ligature applied to the proximal portion of the artery, *i. e.*, as far as possible toward the heart. A little distal to the clamp make an incision in the artery and insert a glass canula and tie it tightly in place. Remove the clamp or ligature and the blood will pass through the canula, and the animal allowed to bleed to death. When the animal is apparently dead, an interesting experiment may be performed by injecting into the artery some normal salt solution of the same temperature as the body and note the reviving effect.

222. The blood obtained as above directed is to be caught in four different vessels and each portion is to be treated as follows : One portion of the blood is to be defibrinated by immediate whipping with some broom straws tied in a small bundle and the fibrin as it collects on the straws is to be saved for future use. The defibrinated blood is also to be reserved for later study. Another portion of the blood is to be collected in a flask and the phenomenon of clotting or coagulation observed. Another portion of the fresh blood is to be mixed with an equal volume of saturated solution of sodium sulphate. And still another portion into a solution of potassium oxalate in the proportion of 1 of the oxalate to 4 of the blood.

223. Test the reaction of blood by pricking one of the fingers behind the nail. Put a drop of the blood on a piece of ordinary litmus paper which has been soaked in salt solution. The substances on which the alkaline reaction depend will diffuse out in a ring around the drop, while the hemoglobin remains in its original position.

224. Place a thin layer of defibrinated blood on a glass slide ; try to read printed matter through it. The blood is too opaque and the print cannot be read, the light is reflected from the corpuscles in all directions, and but little passes through.

225. Place 1 cc. of defibrinated blood in a test tube and add 5 cc. of distilled water, and warm slightly. Note the change of color by reflected and transmitted light. By reflected light it is much darker—almost black, but by transmitted light it is transparent. This constitutes " laky " blood due to the withdrawal of the hemoglobin from the red corpuscles into the water. Test the transparency by looking at some printed matter through this blood as in 224.

226. To 2 cc. of defibrinated blood in a test tube add 5 volumes of a 10% solution of sodium chloride. It changes to a very bright, florid, brick-red color. Compare its color with No. 225.

227. Place a watery solution of defibrinated blood in a dialyzer or parchment tube, and suspend in a vessel of distilled water. After several hours note that no hemoglobin has passed into the water. Test the diffusate for chlorides with silver nitrate and nitric acid. Hemoglobin does not dialyze, although it is crystallizable.

228.　Put a drop of blood on a slide.　Heat it slowly over a flame, so as to evaporate the water.　Then add a small crystal of common salt and a few drops of glacial acetic acid ; put on a cover-glass, and again heat slowly till the liquid just begins to boil.　Take the slide away from the flame for a few seconds, then heat it again for a moment, and repeat this process for two or three times.　Now let the slide cool and examine with the microscope (high power).　The small black or brownish-black crystals of hemin will be seen.　This test is often important in some medico-legal cases where only a trace of blood is available for examination.　If the blood stain be upon a piece of cloth, it may be soaked in a little distilled water and examined by the spectroscope or micro-spectroscope. The liquid may then be evaporated to dryness on the water bath and the hemin test made.　Or perform the hemin test directly on the piece of cloth.

229.　In the blood saved for clotting, note that in a few minutes the blood congeals, and when the vessel is tilted the blood no longer moves as a fluid, but as a solid.　After an hour or so, pale yellow colored drops of fluid—the serum—are seen on the surface, having been squeezed out of the red mass, the latter being the clot and consisting of fibrin.

Note in the clot of horse's blood the upper light colored layer of leucoytes—the buffy coat.　Coagulation is slow in this animal and the red and white corpuscles on account of the difference in their specific gravity have time to separate.

230.　Salted Plasma.　Note that in the flask containing the mixture of blood and sodium sulphate, no coagulation has occurred.　Place some of this fluid in the centrifuge to separate the corpuscles and plasma, or let the mixture stand until the corpuscles sink ; the plasma mixed with the saline solution is known as the salted plasma.

231.　Oxalate Plasma.　Note also that the potassium oxalate blood mixture does not coagulate.　Centrifuge the mixture or let stand until the corpuscles fall, to obtain the plasma. The oxalate precipitates (as the oxalate of lime) the calcium which is necessary for coagulation.

232.　To a portion of the oxalate plasma add a few drops of a 2% calcium chloride solution.　Coagulation results (more quickly at 40°).

the typical ones present 1st time

same as trh says (position)

233. To another portion of the plasma add a little fibrin-ferment prepared by the demonstrator. The fibrin-ferment is prepared as follows : Take fresh fibrin, wash it under a tap with water (best in a piece of cotton) until perfectly colorless. Squeeze out the water and cover the fibrin with an 8% solution of sodium chloride. After a few hours, if the solution is filtered it will show the presence of the ferment.

Another method is : Precipitate some blood serum with about ten times its volume of alcohol. Let it stand for several weeks, then extract the precipitate with water. The water dissolves out the fibrin-ferment, but not the other coagulated proteids.

234. Add a drop of freshly prepared tincture of guaiacum to a small amount of diluted defibrinated blood, and then some hydrogen peroxide or old oil of turpentine. The color changes to blue. This is often used as a test for hemoglobin, but other substances (oxygen carriers) give a blue color under the same conditions.

235. Place some hydrogen peroxide over fresh fibrin in a watch glass ; bubbles of oxygen are given off.

236. Immerse a flake of fibrin in freshly prepared tincture of guaiacum, (5% of pure resin in alcohol) and then immerse the flake in hydrogen peroxide. A blue color is developed, due to the ozone liberated by the fibrin and forming a blue color with the resin. Compare 234.

237. The proportion of the corpuscles to the plasma of the blood may be quite readily obtained by the use of the hematocrit in connection with the centrifuge. The Hematocrit consists of a graduated glass tube 50 mm. in length and 0.5 mm. bore, to receive the blood. The tube is marked by a scale ranging from 0 to 100, the scale being rendered visible by a lens front (prism form). The outer end of the tube fits into a small cup-like depression at the end of the arm, the bottoms of which are covered with the rubber disks, while the inner extremity is held in position by a spring.

To use the Hematocrit in blood examinations proceed as follows :—The rubber tube with mouthpiece at one end is slipped over the end of the Hematocrit, and the latter is filled by suction on the mouthpiece, from a drop of blood obtained by a prick of the finger. The blunt end of the tube is next quickly covered with the finger tip, and the tube is inserted

Turned to full blue

5   Bubbles were given off

6 Pale blue

into the arm in the same manner as adjusting the tubes for micro-organisms. The current is next turned on, and the speed increased gradually to 10,000 revolutions per minute, and thus steadily maintained. for from two to three minutes. The Hematocrit may next be removed and the percentage of red corpuscles is read off from the scale. In health, the volume of red corpuscles is about 50 per cent. One per cent. by volume represents about 100,000 red blood corpuscles, therefore by adding five ciphers to the percentage of volume, it gives the number of red corpuscles in one cb. mm. of blood. Thus in a given case, if the reading were 25, multiply that number by 100,000, and the product 2,500,000 would represent the number of red blood corpuscles in one cb. mm. of blood. The amount of hemoglobin in each corpuscle may be approximately determined, also, by dividing the quantity of hemoglobin ascertained by Fleischl's instrument, by the number of corpuscles determined by means of the Hematocrit.

The white blood corpuscles or leucocytes will be found to occupy a second but much shorter column immediately above the column of red corpuscles, and if leucocytes be present, even though to a very slight degree, it is easily recognized.

XVI

238. Proteid reactions. Dilute 5 cc. of serum with 35 cc. of water. Add a little litmus solution to color and neutralize with 0.2% hydrochloric acid. Is alkali albumin present?

239. To another portion add a little acetic acid and heat.

240. Apply the xanthoproteic reaction.

241. Acidify another portion strongly with acetic acid and add a few drops of a solution of ferrocyanide of potassium.

242. Apply Millon's reagent.

243. Apply Piowtrowski's test (6).

244. To another portion add a little alcohol.

245. Saturate another portion with ammonium sulphate. This precipitates all of the proteids, globulin and albumin. Filter. The filtrate does not respond to any of the tests for proteids.

8 alkali albumin

White ppe shows albumen present
positive

White coagulate
White precipitate.
albumin present (seightly)

White precipitate.

- did as directed but got
, the does to tast that (Ammonium sulphate

246. To another portion of the diluted serum add a little silver nitrate solution. A white, curdy precipitate forms, soluble in ammonia but not in nitric acid. Chlorides are present.

247. Add barium chloride. A white, heavy precipitate insoluble in nitric acid. Sulphates are present.

248. Add nitric acid and molybdate of ammonia and heat. A yellow precipitate indicates the presence of phosphates.

249. Test with Fehling's solution, and boil. Red, cuprous oxide indicates a reducing sugar—dextrose.

250. To a little of the defibrinated blood in a test-tube add a few drops of sulphuric acid. Stir up the solution and note the peculiar odor of blood, intensified by the liberation of traces of volatile acids by the sulphuric acid.

251. Detection of paraglobulin (fibrinoplastin, or serumglobulin). Pass some CO_2 through a beaker of dilute serum for 20 minutes or more. (The CO_2 may be generated by the action of dilute hydrochloric acid upon small pieces of marble in a jar and the gas conveyed to the beaker). Let the precipitate settle. It is paraglobulin. Decant and, after washing with water, dissolve some of it in a little dilute saline solution, use Piowtrowski's test and prove it a proteid.

252. Take equal quantities of blood and ether in a test-tube. Shake thoroughly and let the ether separate. Then pour the ether into a watch-glass or evaporating dish and when evaporated examine for globules of fat.

253. Evaporate a little blood to dryness in a crucible or evaporating dish. Raise the temperature to red heat to convert the blood to ash. When cool add a little nitric acid, heat, dilute with water and filter. Make the following tests with the filtrate :

254. To a small portion of the filtrate add a little sulphocyanide of potassium. A red color indicates iron.

255. To another portion add a little ammonium molybdate solution. A yellow precipitate, after allowing the mixture to stand for some time, indicates phosphates.

256. To another portion add a little silver nitrate solution. A white, cloudy precipitate indicates chlorides.

257. Examination of blood with a spectroscope. With a small direct vision spectroscope focus on the sky or bright light until the spectrum shows clearly. Narrow the slit un-

7 Positive
8 Positive
9 Positive
, Good strong odor.

- - Positive.

did this to obtain material for
owing experiments

Red Positive
- Yellow Positive

Positive little cloudy.

Demonstrated by Instructor in
arge.

til the spectrum is as distinct as it can be made. Hold the spectroscope so that the red is at the left of the field. Dip a wire into some water, and then into some salt or sodium carbonate, and hold it in a flame of a fish-tail burner. Note the change in the spectrum.

258.   Arrange the apparatus with the aid of a demonstrator, so that the spectroscope, gas-flame and substance to be examined, are in their proper relations. Half fill the vial or test-tube with defibrinated blood. Nothing can be seen until the blood is properly diluted. Continue diluting until two bands of oxyhemoglobin appear in the spectrum. Note their position, and which one disappears first when the solution is diluted far enough.

259.   Add a drop or two of ammonium sulphide solution or Stokes' fluid to reduce the oxyhemoglobin. Note the result. Stokes' fluid is prepared by 2 grams ferrous sulphate and 3 grams of tartaric acid. These are mixed and preserved dry for use. When required, add 100 cc. of water and enough ammonia to make slightly alkaline.

260.   Pass some illuminating gas through some blood for a considerable time. Examine with a spectroscope. Add a drop or two of ammonium sulphide or Stokes' Fluid. Compare this with 257.

PART II

Experimental Physiology

XVII

Each dissection is to be carefully demonstrated to one of the instructors before beginning the dissection of a new part.

261. DISSECTION OF FROG'S HEART. With a pair of strong scissors and forceps cut through the pectoral girdle. Remove the sterum and expose the heart, cut carefully through the pericardium and note the division of the heart into ventricle, auricles, and truncus arteriosus. (Do not remove the heart until the vagus nerve has been dissected).
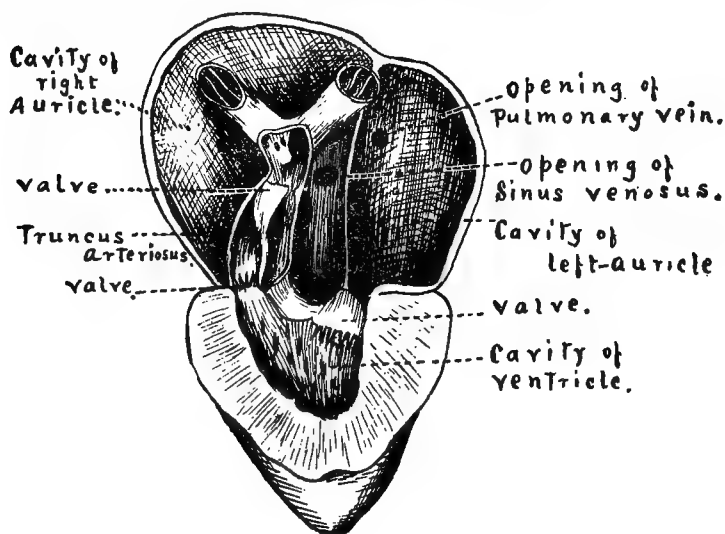

FIG. 1

Fig. 1. Dissection of the frog's heart showing the relationship of the cavities and the principal blood vessels.

Raise the apex of the ventricle slightly and note a delicate band of connective tissue binding the ventricle to the body, (the frenum). The ventricle is of a conical shape and is usually of a paler color than the auricles. It has thick walls.

The auricles are two in number although externally the division is not easily apparent. The right is larger than the left and both are usually engorged with blood. The walls of the auricles are thin.

The truncus arteriosus is a cylindrical tube somewhat swollen as it lies upon the auricles. The truncus soon divides into two arches, one passing to the right, the other to the left. Each arch soon splits into three vessels, the carotid, for the head, the pulmo-cutaneous, carrying the venous blood to the lungs and skin to be oxygenated, and the aorta, which curves around to the back to meet its fellow with which it unites to form the descending aorta. Lift up the ventricle and make out the following structures : The right and the left superior vena cava, bringing back blood from the head and upper extremities ; the inferior vena cava, appearing just above the liver ; the sinus venosus, (practically a fusion of the venae cavae), the chamber into which the three cavae open. The sinus in turn communicates with the right auricle.

Carefully slit the heart lengthwise into ventral and dorsal halves. In the ventricle note the comparatively small size of the cavity and the thick walls ; note the two openings in the ventricular cavity—one from the auricles, guarded by auriculo-ventricular valves, the other continuous with the truncus arteriosus ; note in the truncus at its base near the ventricle three small semilunar valves, also a longitudinal fold or so-called spiral valve. The swollen portion of the truncus is known as the pylänguim ; the distal portion formed by the fusion of the aortic arches is known as the synangium.
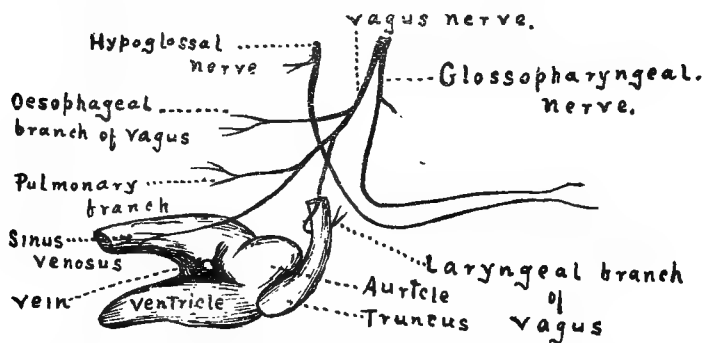

FIG. 2

Fig. 2. Lateral aspect of the heart, showing its principal parts and the distribution of the branches of the vagus nerve.

262. DISSECTION OF THE VAGUS NERVE. Introduce a glass rod into the frog's throat to distend the parts. Beginning

66

at the angle of the mouth, remove the skin between it and the
arm and tympanum. The projection formed by the articu-
lation of the lower and upper jaws may be cut off. Remove
the arm. Dissect away the muscles and expose the scapula;
this in turn may be tilted to one side or removed. In front of,
and partially under, the scapula nerves may be seen. Two
nerves will be found lying close together and accompanied by
a blood vessel. The first of these nerves is the glossophar-
yngeal, the second the vagus with its branches. Follow both
nerves toward the cranium and note that both glossopharyngeal
and vagus emerge from the cranium through the same foramen.

Dissect the glossopharyngeal distally and note that it
supplies the tongue.

Dissect the vagus noting that it gives off a branch to the
oesophagus, then a smaller one the laryngeal curving around
the aorta to supply the larynx, and finally branches to the
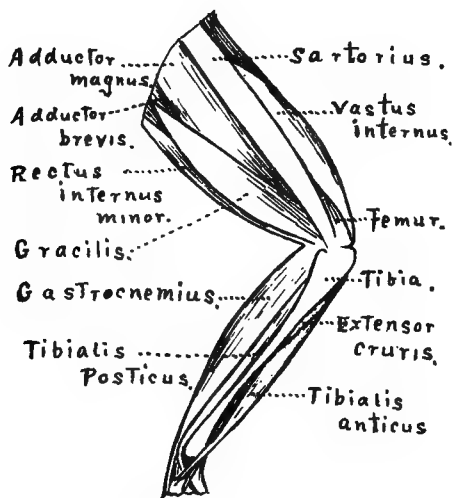lungs and heart.

FIG. 3

Fig. 3. Ventral aspect of the superficial muscles of the left leg of the frog.

263. DISSECTION OF MUSCLES OF FROG'S THIGH AND LEG.
Ventral Aspect. Remove the skin from the ventral aspect of
the leg and expose the superficial muscles.

Sartorius, a long narrow muscle crossing the thigh obliquely from the outer to the inner side. It arises from the iliac symphysis below the acetabulum and is inserted into the inner side of the head of the tibia.

The *Adductor Magnus* is a large muscle lying along the inner border of the *sartorius* but passing beneath it at its distal end. Its origin is from the pubic and ischial symphyses, and the muscle passes under the *sartorius* to be inserted into the distal third of the femur.

The *Adductor Longus* is a long, thin, narrow muscle lying along the outer side of the *adductor magnus* and often completely hidden by the *sartorius*; its origin is from the iliac symphysis beneath the *sartorius* and unites a little way beyond the middle of the thigh with the *adductor magnus*.

The *Rectus Internus Major* or *Gracilis*, is a large muscle lying along the inner side of the *adductor magnus* and of the *sartorius*. Its origin is from the ischial symphysis and it is inserted into the head of the tibia.

The *Rectus Internus Minor* is a narrow, ribbon-like muscle passing along the inner or flexor margin of the thigh; it arises from a tendinous expansion connected with the ischial symphysis and is inserted into the inner side and just below the head of the tibia.

264. Dorsal Aspect of the Thigh. The *Triceps Extensor Femoris* is the great extensor muscle of the thigh; it arises by three distinct origins from the ilium and acetabulum and is inserted into the tibia just below its head.

The *Rectus Anticus Femoris* is the middle division of the triceps; it arises from the ventral border of the posterior third of the ilium in front of the acetabulum; about half way down the thigh it joins the next division.

The *Vastus Internus* is the ventral head of the triceps and lies between the *sartorius* and the *rectus anticus*. It arises from the ventral and anterior border of the acetabulum.

The *Vastus Externus* is the dorsal head of the triceps. It arises from the posterior edge of the dorsal crest of the ilium and joins the other two divisions of the triceps at about the junction of the middle and distal thirds of the thigh.

The *Gluteus* lies in the thigh between the *rectus anticus*

and the *vastus externus*.   It arises from the sacrum and is attached to the femur.

The *Biceps* is a long, slender muscle arising from the crest of the ilium just above the acetabulum.   It lies in the thigh along the inner border of the *vastus externus* and is inserted by a flattened tendinous expansion into the distal end of the femur and the head of the tibia.
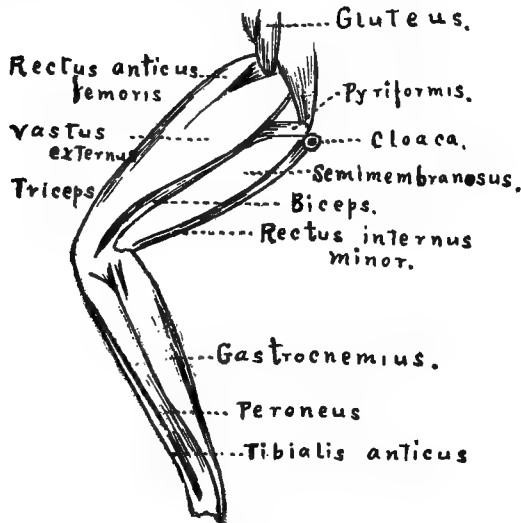

FIG. 4

Fig. 4.   Dorsal aspect of the superficial muscles of the left leg of the frog.

The *Semimembranosus* is a stout muscle lying along the inner side of the *biceps*, between it and the *rectus internus minor*. It arises from the dorsal angle of the ischial symphysis just beneath the opening of the cloaca and is inserted into the back of the head of the tibia.   There is an oblique line of tendinous intersection running obliquely through its middle.

The *Pyriformis* is a slender muscle which arises from the tip of the urostyle, passes backwards and outwards between the *biceps* and the *semimembranosus* and is inserted into the femur at the junction of its proximal and middle thirds.

265.   VENTRAL ASPECT OF THE DEEP MUSCLES OF THE THIGH.   The *Semitendinosus* is a long, thin muscle which arises by two heads, an anterior one from the ischium close to the

ventral angle of the ischial symphysis and the acetabulum; and a posterior one from the ischial symphysis. The anterior head passes through a slit in the *adductor magnus* and unites with the posterior head in the distal third of the thigh. The tendon of insertion is long and thin and joins that of the *rectus internus minor* to be inserted into the tibia just below its head.

The *Adductor Brevis* is a short wide muscle lying beneath the upper end of the *adductor magnus*. It arises from the pubic and ischial symphyses and is inserted into the proximal half of the femur.

The *Pectineus* is a smaller muscle lying along the outer (or extensor) side of the *adductor brevis*. It arises from the anterior half of the pubic symphysis in front of the *adductor brevis* and is inserted like it into the proximal half of the femur.

266. DORSAL ASPECT OF THE DEEP MUSCLES OF THE THIGH. The *Ilio-psoas* arises by a wide origin from the inner surface of the acetabular portion of the ilium; it turns around the anterior border of the ilium and crosses in front of the hip joint, where for a short part of its course it is superficial between the heads of the *vastus internus* and of the *rectus anticus femoris*; it then passes down the thigh beneath these muscles and is inserted into the back of the proximal half of the femur.

The *Quadratus Femoris* is a small muscle on the back of the upper part of the thigh; it arises from the ilium above the acetabulum and from the base of the iliac crest; it lies beneath the *pyriformis* and is inserted into the inner surface of the proximal third of the femur, between the *pyriformis* and the *ilio-psoas*.

The *Obturator* is a deeply located muscle which arises from the whole length of the iliac symphysis and the adjacent parts of the iliac and pubic symphyses and is inserted into the head of the femur close to the *gluteus*.

267. MUSCLES OF THE TIBIAL PORTION OF THE LEG. The *Gastrocnemius* is the large muscle forming the calf of the leg; it has two heads of origin, the larger of which arises by a strong flattened tendon from the flexor surface of the distal end of the femur; while the smaller head which joins the main muscle about one-fourth of its length below the knee, arises from the edge of the *triceps extensor femoris* where it covers the knee. The muscle is thickest in its upper third and tapering posteriorly ends in the strong Tendon of Achilles,

which passes under the ankle joint, being much thickened as it does so and ends in the strong plantar fascia of the foot.

The *Tibialis Posticus* arises from the whole length of the flexor surface of the tibia ; it ends in a tendon which passes around the inner malleolus, lying in a groove in the lower end of the tibia and is inserted into the dorsal surface of the astragalus.

The *Tibialis Anticus* lies on the extensor surface of the leg ; it arises by a long thin tendon from the lower end of the femur and divides about the middle of the leg into two bellies which are inserted into the proximal ends of the astragalus and calcaneum respectively.

The *Extensor Cruris* lies along and is partly covered by the *tibialis anticus.* It arises by a long tendon from the condyle of the femur and runs in a groove in the upper end of the tibia and is inserted into the extensor surface of the tibia along nearly its whole length.

The *Peroneus* is a stout muscle which lies between the *tibialis anticus* and the *gastrocnemius.* It arises from the distal end of the femur and is inserted into the outer malleolus of the tibia and the proximal end of the calcaneum.

268. DISSECTION OF THE SCIATIC NERVE. Expose the muscles of the dorsal aspect of the thigh, carefully separate the *biceps* and *semimembranosus* ; closely applied to the deeper

Nerve muscle preparation

FIG. 5
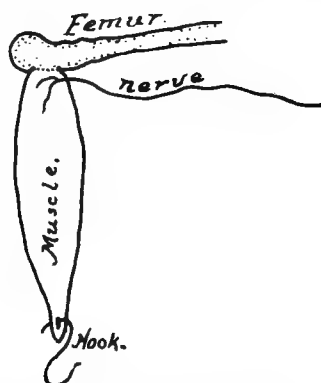
margin of the *biceps* will be found the sciatic nerve accompanied by a blood vessel. Carefully follow the nerve toward the body noting its passage between the *pyriformis* and the head of the *biceps*. Follow the nerve up to its connectiôn with the lumbar region of the spinal cord.

Return to the middle of the thigh and note that the nerve sends off a branch which passes along the entensor side of the tibia along the *peroneus* and beyond. Follow the sciatic and note that at the knee joint it again divides, one branch going . to the *gastrocnemius* and the other to the *tibialis posticus* muscle. The *sciatic* nerve, *gastrocnemius* muscle and a portion of the femur comprise a nerve-muscle preparation.

XVIII

269. Place a frog on its belly and note the movements of the caudal lymph-hearts. They are situated between the hip-joint and the median line in a slight depression. The contractions of these hearts are usually visible through the skin, but are seen more distinctly if the skin is removed without injury to the heart.

Later, note that the lymph-hearts cease to beat after the destruction of the caudal portion of the myel (spinal cord).

270. Pith the frog. This is accomplished by severing the brain from the myel with a thin bladed knife at the point where the cranium articulates with the atlas. A slight depression will be felt at this point, which will serve .as a guide for the operation. The frog may be firmly held if wrapped in one corner of a towel.

271. After pithing, lay the frog on its back and cut through the skin on the mid-line, and from the middle of this cut make lateral incisions through the skin. Raise up the end of the sternum and cut, a little to one side of the mid-line, through such parts as may be necessary to expose the heart. Pin the parts out on the side and note the heart beating with some force and regularity. Count the number of heart beats per minute. Pinch up the pericardium with a pair of fine forceps and remove it from the heart. Tilt up the apex of

42 Per sec

the ventricle and note a small band of connective tissue passing from its dorsal surface to the adjoining wall of the pericardium. Seize this band with the forceps and divide it between the forceps and the pericardial wall. Connect the apex of the ventricle with a heart lever and take a tracing of the heart beat upon the kymograph (revolving drum). Paste the tracing in your notes. Lift up the apex of the ventricle, by means of the band already described, and with a sharp pair of scissors cut through the right and left aortae, the pre and post caval veins, and the surrounding tissue, taking care not to injure the sinus venosus. Place the heart in a watch-glass, moistening occasionally with normal saline solution.[1] The beats will not be interrupted at all, or for a very short time only.

272. Watch the beating of the heart. Do the auricles and ventricle contract simultaneously? What are the number of beats per minute ? Compare with 271.

273. Lift up the apex of the ventricle, and with the scissors cut off the apex at the upper third of the ventricle. Watch the separated portions. Is there any difference in the beating ?

274. With the scissors separate the two auricles from each other, letting the attached portion of the ventricle remain to each auricle. Do they continue to beat ?

275. The same frog, if it has been kept in a moist place, may be used for the following cilia experiment : Place the frog upon its back, and cut through the lower jaw, along the midline, continuing the incision down the oesophagus as far as the stomach. Pin the parts back and moisten the mucosa with normal salt solution, if it is at all dry. Place a small, thin piece of cork upon the mucosa just below the orbits, and note that the cork is carried toward the stomach by the cilia. Warm a little of the normal salt solution to 30° C., and repeat the experiment. Apply heavier bits of substance to the mucosa, and note if their positions are changed. Apply to the strip of mucosa a few drops of a saturated solution of chloretone and note whether the motion of the cilia is affected or not. With a scalpel scrape some of the mucosa and examine the ciliated cells in saline solution under the microscope.

---

[1] Made by dissolving 6.5 grams of sodium chloride in one liter of distilled water.

2 18 Per min

Auricles 1st then ventricles.

Yes

No

Cilliated movement fine

276.   If the caudal lymph-hearts are still beating, pass a tracer or piece of wire down the spinal canal to destroy the myel.   If thoroughly destroyed the lymph-hearts will cease to beat.

XIX

277.   The circulation of blood.   This may be shown very nicely in the delicate external gill filaments of the *Necturus*, or in the tail of a tadpole, or in the web of a frog's foot which does not contain too much pigment.   The animals should be injected with a few drops of a 1% solution of curare, in order that they may not move, and arranged upon the stage of the microscope, so that the parts to be examined may come clearly into the field of vision.   Precautions should be taken against drying, by keeping the animal well surrounded with moist cloth or absorbent cotton.

278.   If the frog is more convenient, prepare it by destroying the brain and injecting the curare under the skin of the back.   Place the frog on its belly on the frog board and pin out the digits so that the web will be slightly on the stretch. Keep the parts moist.   Put a very *small* drop of water upon the web, and cover it with a triangular piece of cover-glass, being careful that it does not cut the digits and that no fluid flows over its surface.   Examine first with a low power, and then, if possible, with a high power.

279.   Note the course of the blood from the arteries to the veins.   Arteries may be distinguished from veins by the fact that the blood corpuscles scatter to enter the capillaries diverging from the artery, while in the veins the corpuscles accumulate from the capillaries converging to form the vein.   A slight pulsation may sometimes be observed in the smaller arteries.

280.   Note the greater velocity of blood in the arteries than in the veins ;   the individual corpuscles cannot, perhaps, be made out in either.

281.   Note the axial and peripheral zones in the arteries and veins ;   the peripheral zone is small and under a low power appears free from corpuscles ;   under a high power a few leuco-

.

. .

positive

positive

Could not see under high power

saw OK under low

cytes may be seen in the peripheral zone, if the current is not too rapid ; in that of the veins a few leucocytes and occasionally a red one will be seen moving along comparatively slowly.

282. Note the passage of the corpuscles usually in single file through the capillaries.

283. Note the elasticity of the red corpuscles, observing the way in which they bend and later regain their normal form.

284. Study of inflammatory conditions.   Remove the cover-glass and absorb the fluid on the web ; touch the middle of the web with the tip of a glass rod that has been dipped in creosote (or a 2% solution of croton oil in olive oil) leaving a minute drop on the web. Put on a cover-glass as before and examine with the microscope.   If not successful with the web, try the tongue or mesentery.

285. Note the dilation of the arteries, the more distinct appearance of the capillaries, and the enlargement of the veins, accompanied by a quickening of the current.

286. Note a little later, the slowing of the current, the vessels remaining dilated.

287. Note that the leucocytes increase in number in the peripheral zone of both arteries and veins ; in the latter the leucocytes begin to cling to the sides, temporarily at first and then permanently.   In the capillaries the leucocytes and, less frequently, the red corpuscles stick to the capillary walls, partially or completely blocking the way. Later stagnation may set in and there is then the appearance of the gradual obliteration of the outlines of the corpuscles.

288. Note the migration of the leucocytes from the capillaries and veins.   This occurs when the circulation becomes slow.   Watch, at intervals of 10 minutes, some particular leucocyte adhering to the wall of a capillary or vein.

289. Note the diapedesis of the red corpuscles from the capillaries, seen to the best advantage in those capillaries in which the current has almost ceased.

290. Note that the above effects are local, are of greatest intensity in the spot touched, they extend some distance around the spot, but the circulation in the rest of the web is normal.   If the injury has not been too severe, the circulation may become re-established in the stagnated spots, and the inflammatory appearances disappear.

~2 Dot times appeared single
then in bundles.

3 positive

5 — OK.

~6 current slowed

~7 stagnation first phase.

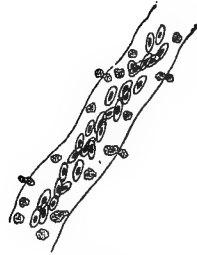_ spot touched has greatest
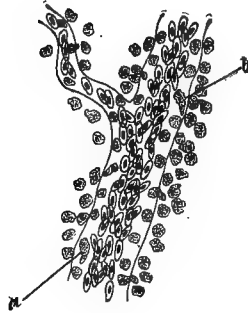intensity .

FIG. 6

FIG. 7

FIG. 8   FIG. 9

Figs. 6 to 9. Fig. 6.—Leucocytes sending forth processes which penetrate the wall of the vessel. Fig. 7.—Leucocytes partly through vessel wall, showing constriction in centre. Fig. 8.—Leucocytes after penetrating wall regain former shape. Fig. 9.—Appearance of vessel and surrounding tissue after diapedisis has gone on for some time. (After Craig).
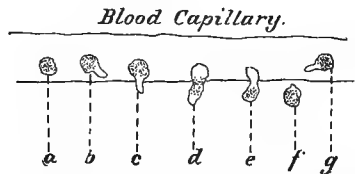
Blood Capillary.

Fig. 10.—Diagrammatic Representation of the Manner in which a Leucocyte Traverses the Wall of a Capillary Blood-Vessel. (After Craig). *a*, Leucocyte before penetrating ; *b*, leucocyte sending off process and granules beginning to withdraw to farther end of cell ; *c*, leucocyte partly through wall, granules at upper end of cell ; *d*, granules passing through the wall ; *e*, granules arranged in the portion of the leucocyte farthest from vessel ; *f*, leucocyte, after penetration, resuming its original condition ; *g*, leucocyte swept from wall, showing retention of the clear penetrating process.

291. Pin out the two horns of the tongue and observe that under the microscope. The tongue is at first pale but soon becomes reddened as the vessels become filled with blood. With a low power the peripheral zone in the arteries and veins may probably be seen better here than in the web.

292. Place the frog on its back, cut through the skin and muscles on one side and draw out the mesentery and pin out a loop of it under the field of the objective and observe the circulation. The inflammatory phenomena can be well seen in this preparation or that of the tongue. (291).

XX

293. Experiments in reflex action. Pith a frog and place it on its belly. Note the position of its fore and hind limbs. Note the position of the head as compared with a normal frog. Are there any respiratory movements at the nostrils or throat?

294. Pull, very gently, one of the hind-limbs into an extended position and then let go. Does it return to its former location?

295. Gently tickle one flank with a feather or blunt needle. Is there any contraction of the muscles?

296. Pinch the same spot sharply with a pair of forceps. Is there any movement of the leg of the same or opposite side?

297. Pinch the skin around the anus with a pair of forceps. What is the effect upon the legs?

298. Place the frog on its back. Does it make an effort to get into a natural position? Does it show any sense of equilibrium?

299. Pass a hook through its lower jaw and hang it to the ring of a retort stand. How do the hind-limbs behave?

300. Pinch very gently the tip of one of the toes; what is the effect?

301. Fill two glasses, one with dilute sulphuric acid, the other with water. Raise the glass containing the acid, until the acid just touches the tip of the toes. Is the foot withdrawn? If so, raise the second glass and let the foot be immersed in it, to wash off the acid.

291 Very good — Position.

92 positive.

93. Limbs stretched out slightly
and a reflex action

4 Yes.
5 Yes
96 Opposite leg moved
7 Leg on drawn up.
8 Yes

9 Draw them up

10 Flexion

301 Yes

302. Cut a small piece of filter or blotting-paper, moisten it with strong acetic acid and place it on the flank of the animal. What is the effect upon the leg? Put the piece of paper upon the opposite flank and hold the leg so as to prevent it from moving. Is there any action of the opposite leg?

303. Place similar pieces of paper upon different portions of the body. Note any variety of movements and what seems to be their purpose.

304. Remove the frog from the hook and plunge it in a basin of water. This will wash off the acid. Does the frog make any movements in the water ? Does it float ?

305. Open the abdomen and draw out a loop of intestine. Expose the heart and while its movements are under observation, strike or pinch the intestine ; the heart is temporarily arrested. If you divide the vagi or completely destroy the oblongata and repeat the stimulus, the arrest does not occur. Or instead of the intestine you may employ strong stimulation of a limb by the sudden tightening of a string around it. The heart will stop, and the body of the frog will become inert and flaccid, and will not respond to cutaneous stimuli. The bulbo-spinal axis is in a state of " shock ". [Waller]. (An unpithed frog may be required for the success of this experiment).

306. Inject 3 to 5 minims of 0.2% strychnine solution under the skin of the frog's back. Let it remain for a few minutes and then note the effect of the slightest stimulus, such as jarring the table upon which it lies. Then give 10 to 20 minims of 10% chloral hydrate with a pipette. Make sure that the fluid reaches the stomach. Note if there is any effect upon the convulsions.

307. With a tracer or piece of wire destroy the myel, the convulsions cease. Try any of the preceding stimuli upon the frog now and note the result.

308. Make a nerve-muscle preparation of one of the hind limbs. Dissect away the skin and muscles upon the dorsal aspect of the leg, until the sciatic nerve is exposed, leaving it connected with the lumbar plexus. Denude the femur of its muscles, using the greatest care not to injure the sciatic nerve. Keep the nerve moist with normal salt solution. Pass a copper hook under the sciatic nerve and hang to a

302 ...

303 neg —

'04 ...iks makes slight movement

'5 Result as stated.

6 Jumps Increase convulsions.

7 They are distroyed ...inutes, no effect

8 muscle contracts

tripod. Tilt the tripod so that the leg may come in contact with one of the iron supports. If the tripod has been painted, scrape the paint off. What happens when the contact is made? This is known as Galvani's experiment.

309. Make another nerve-muscle preparation of the other hind limb, but cut the sciatic nerve as near to the myel as possible and separate the leg from the body at the femoro-pelvic joint. Remove the skin as far as the foot. With the forceps crush the gastrocnemius muscle near the tendon of Achilles. See that the end of the nerve is cut off squarely. With a small brush or thin glass rod lift the nerve very carefully in such a way that its cross-section may fall upon the injured portion of the muscle. This stimulates the nerve and causes a contraction of the muscle due to the so-called demarcation currents.

XXI

310. INDUCTION MACHINE OR INDUCTORIUM. Principle of action : If portions of the wires forming two separate cir-
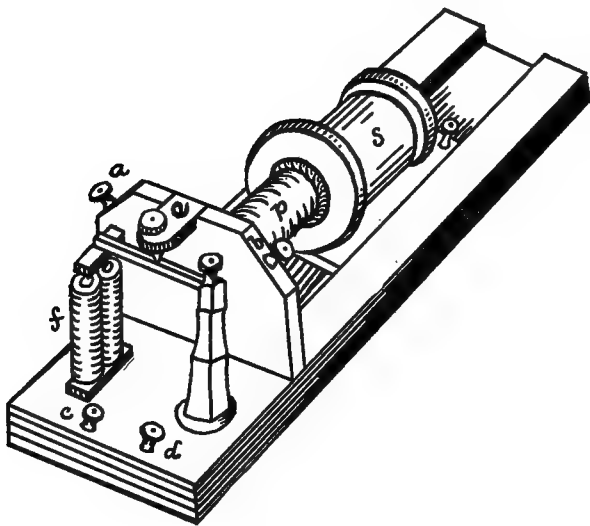

FIG. 11

Fig. 11, Induction coil. *ab*, binding posts for single induced currents ; *cd*, binding posts for interrupted currents ; *e*, post connecting Neef's hammer with the primary coil; *f*, electro-magnet ; *p*, primary coil ; *s*, secondary coil.

muscle contracted

cuits be placed parallel to each other, as in the case of the planes of two spirals or coils of the inductorium, the one wire primary (P), being connected with a source of electricity (battery), the other, the secondary (S), being simply a closed circuit ; whenever the P circuit is closed (made), or is opened (broken), currents will at those moments be induced in the S circuit.

The *make* induction current flows in the S circuit in a direction *opposite* to that of the P circuit : whilst the *break* induction current flows in the *same* direction as the original battery current.

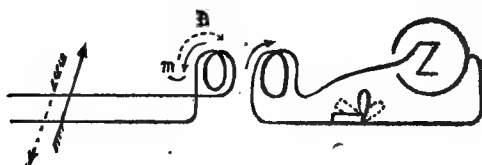These induction currents are of very short duration.

FIG. 12

Fig. 12, showing the direction of the make and break currents. In the secondary circuit the direction of the break current is shown by the dotted line and arrow, and the make by the continuous line.

Place the induction machine lengthwise in front of you on the table with the interrupter turned to the right. In the DuBois Reymond type the wires are wound into two separate coils ; the P coil which is supported by a wooden upright attached to the base of the instrument is composed of relatively thick wire, while the S coil mounted upon a sliding foot is composed of very thin wire, in this case invisible, as it has a protective covering of vulcanite.

The parallelism of the wire in the two coils is maintained so long as the axes of the coils coincide. The successive turns of the wire in each coil are also practically parallel to each other. The P coil is provided with a core of soft iron wire which magnetizes when a current passes in the surrounding wire, an electro-magnet being thus produced.

The electrical field produced by the coil is greatly intensified by this core, and the effect on the S coil is correspondingly increased. The nearer the S coil is to the P coil, the more powerful will be the induction currents.

The electromotive force of the currents in the secondary bears a direct relationship to the primary. Thus, if there are 200 turns in the P and 6000 in the S, the electromotive force of the induction currents would be about thirty times as great as the primary, independently of the influence exerted by the iron core.

311. CONNECT THE SECONDARY CIRCUIT OF THE INDUC-TORIUM. It is well to do this first in all cases. Fasten a key to the table close to the left end of the machine as it now rests upon the table, and connect the binding screws of the S coil with those of the key by means of two wires, so that when the key is closed the S circuit is thereby also closed. This is the *short circuiting key* in the secondary circuit.
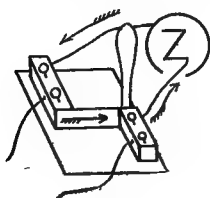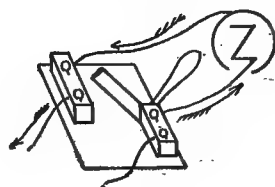
FIG. 13   FIG. 14

Fig. 13—Key closed.   Fig. 14—Key open.

Now attach the long circuit wires by means of which the connection is to be established, with the seat of stimulation, *i. e.*, attach the electrodes by their metal tags to the other pair of binding screws of the key.

312. CONNECT THE P COIL FOR SINGLE INDUCTION CUR-RENTS. Place the battery upon the table near the right hand end of the coil and attach a key to the table close to it ; keep the key open. In making the connections always begin at the battery, and follow the direction the current will take.

Connect the C (carbon) pole of the cell to the key by a wire, then wire the other side of the key to the top binding screw *a*, fig. 11, of the P coil, wire *b* to the zinc pole of the cell.

Withdraw the S coil to the end of the scale and let one co-worker hold the electrodes to the tip of the tongue or to a nerve muscle preparation, whilst the other makes the trials.
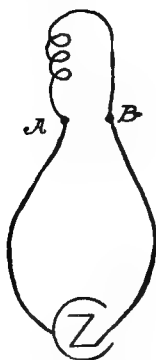
FIG. 15

Fig. 15—*a*, *b*, binding posts of primary coil.

Make and Break the P Circuit with the Key 1. Fig. 16. Do this smartly once or twice only and after each trial push the S coil 1 centimeter towards the P coil. Let the co-worker indicate when he feels the " shock " and whether he does so at closure or opening. Note the position of the coil as soon as the *minimal* break shock is felt ; it is perceived first. Proceed with further trials until the make shock is also felt. Read off the position of the S coil. It is considerably nearer to the P coil. The *break* shock is the *stronger* of the two. Continue the approximation of the S coil by short distances to the P coil. The shocks will be stronger each time until finally unbearable.

The strength of a stimulus can therefore be varied by changing the relative position of the S coil. It may approximately be assumed to change inversely with the square of the distance between the two coils.
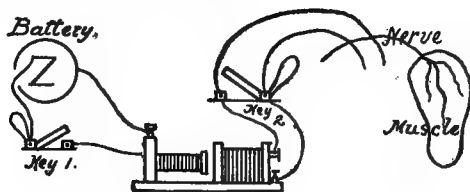

FIG. 16

Fig. 16. Apparatus as set up for make and break shocks.

Remove the core of soft iron from the P coil. Find the . minimal shocks for break and make shocks and compare with the readings in the previous experiments.

Next take the S coil out of the slide and place it end on and close up to the P coil. Whilst making and breaking the P circuit turn the S coil so that its axis shall be ultimately set at right angles to that of the P coil. The shocks will rapidly diminish and finally disappear as the position of the S coil is changed.

Explanation : When the battery current at closure of circuit is rising in strength in the primary, an opposing induction current is thereby generated in the P coil itself, which retards the battery current from attaining its full strength as soon as it otherwise would, and of course the effect upon the S coil is not so sudden a one.

On breaking the P circuit an induction current is likewise generated which has the *same direction* as the *disappearing battery current*, and consequently it retards change of the electrical condition but does not interfere much with the suddenness of the subsequent drop in potential and therefore the effect upon the S coil is greater than at closure.

313. INTERRUPTED SHOCKS. Detach the wires from *a* and *b* and transfer them to the binding screws *c* and *d*. Adjust the top contact screw *e* so that it touches the spring lightly. Fig. 11.
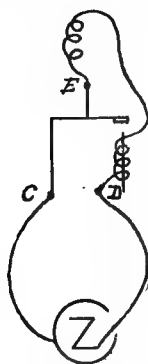

FIG. 17

FIG. 17, *C*, binding post ; *E*, primary coil ; *D*, electro-magnet and binding post.

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On closing the P circuit this spring oscillates automatically opening and closing the P circuit, and a succession of induction currents is generated in the S coil. The rate of their occurrence depends upon the length of the spring.

Explanation : The current from the battery flows through the binding screw *d*, up the pillar. through the spring, up through the top contact screw *e* to the P coil, and thence round the electro-magnet *f* and back by the base of *c* to the battery. Fig. 11.

When the current flows round the circuit, *f* is magnetized and draws down the spring thus breaking the top contact. Upon this the current stops flowing, the magnet ceases to act, the spring is released and again makes contact with *e* and so the circuit is re-established and the cycle begins anew.

As the break shock is always the stronger of the two, it follows that if these shocks are passed through a tissue for some time that polarization effects will be set up. Ordinarily they are employed for a short time only, and this effect can be disregarded.

314. ELECTROLYSIS OF POTASSIUM IODIDE. An interesting example of electrolysis is seen in the decomposition of potassium iodide. Dip a small piece of filter paper in starch paste to which about 5% of potassium iodide has been added and lay the paper over the electrodes. Make and break the circuit using the single induced current. Iodine is set free at the anode and turns the starch blue, forming the iodide of starch. This method may be used to determine which is the anode. The direction of the current in the secondary coil of the inductorium may thus be recognized. (Porter.)
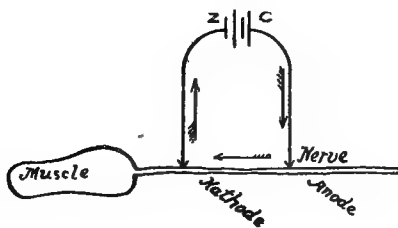

FIG. 18

A make contraction starts from the kathode, a break contraction from the anode.

This experiment also shows that the current passes in opposite directions in make and break.

315. THE BREAK EXTRA-CURRENT. When a galvanic current traversing the primary coil of an induction machine is made or broken, each turn of the wire exerts an inductive influence on the others. When the current is made the direction of the extra current is against, or in an opposite direction to that from the battery, but at break it is in the same direction as the battery current.
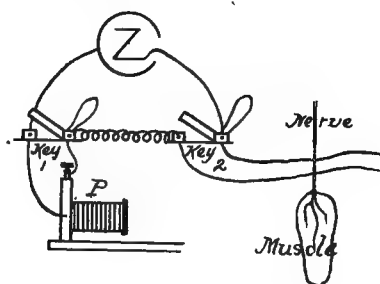

FIG. 19

FIG. 19. The Break-Extra Current.

Apparatus : Battery, two keys, wires, primary coil of induction machine. Arrange the apparatus according to the diagram. Fig. 19. Both keys and the coil are in the primary circuit, the keys being so arranged that either the primary coil, P, or the electrodes attached to key 2 can be short circuited. Test either by electrodes applied to the tongue or by means of a nerve muscle preparation. Close key 1, thus short-circuiting the coil. Open and close key 2. There is very little effect. Open key 1, the current passes continuously through the primary coil. Open key 2 ; a marked sensation is felt, due to the break-extra current.

316. RHEOCORD. The rheocord consists of a brass or German silver wire (in this case 20 meters in length), placed along a square board, with its ends connected with binding posts. On the wire is a " slider " which can be pushed along the wire as desired. On account of the difference in potential

of the two poles of the cell, the potential through the wire will fall uniformly from the anode to the kathode. The difference of potential between post 0 and post 1 will be practically one-tenth the electromotive force of the element. Connect the battery (or two) through a key with the rheocord, at posts 0 and 1. Arrange the electrodes from the "slider" post to come in contact with the muscle. Close the key and note the effect. Move the "slider" along the wires and note the effect.

An electric current can be graduated by changing the number, arrangement and size of the cells, or by using a rheocord to divide the current itself, the battery remaining constant.

317. UNIPOLAR EXCITATION. Set up the battery and inductorium to give single shocks, and at first attach only one wire to the secondary coil. Prepare a nerve-muscle preparation and place it upon a dry glass plate, putting the single wire from the secondary coil under the nerve. Open and close the key in the primary circuit ; no contraction occurs. Insert a second wire in the other binding post of the secondary coil and attach its other end to a gas pipe thus connecting with the earth. A contraction will now occur on opening or closing the primary circuit. In the latter case, the amount of current which passes through the earth and the glass plate is sufficient to stimulate the nerve. The short-circuiting key in the secondary circuit is therefore used in most experiments in order to avoid excitation of the nerve in this way.

318. POLARIZATION OF ELECTRODES. If a pair of clean platinum wires be immersed in water, and a current sent through them for a time, it is found that both of the platinum terminals become covered with bubbles of gas. The one in connection with the negative pole of the battery is covered with hydrogen, and the other with oxygen. Upon the removal of the battery and connection of the electrodes with a galvanometer, a current will be demonstrated having a reverse direction to that first induced. This condition at the electrodes is known as polarization of electrodes.
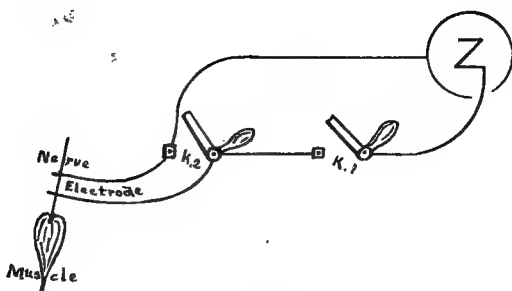
FIG. 26

FIG. 26. Polarization of electrodes.

If a piece of fresh animal tissue connects the pair of wire electrodes, instead of the solution, the same polarization occurs. Chemical changes occur where the wires touch the tissue which can act in the reverse manner, and set up a small current if the battery be removed and the electrodes connected by a conductor. This acts as a source of fallacy in many experiments and is of much importance when a very excitable tissue, such as a nerve, is dealt with. The following experiment will illustrate polarization.

Arrange the apparatus as shown in fig. 26, open the key, k2 and close the key, k1. The current is sent through the nerve and will polarize it. There is no contraction of the muscle while the current is passing. After one or two minutes, open k1, and then rapidly close and open k2, when contractions will occur, which are due to the closing and opening of the small current set up by the polarized electrodes. The contractions diminish quickly in amount as the nerve becomes depolarized.

In order to avoid polarization effects, special forms of electrodes may be used. These are known as unpolarizable electrodes and usually consist of a glass tube containing a saturated solution of zinc sulphate. The electrode end of the tube is filled up with a pad of china clay or camel's hair brush, upon which the nerve is laid ; the other end of the tube is fitted with a binding post attached to a zinc wire which dips into the zinc solution. The electrodes in the moist chamber are an example of unpolarizable electrodes.

319.   Each student is to have a frog, which is to be pithed and have its brain and myel destroyed by passing a tracer or seeker through the spinal canal.   The legs are to be used for nerve muscle preparations.   Dissect one leg for the first series of experiments, and reserve the other leg for the second series. Begin the dissection upon the dorsal aspect of the leg, removing the skin and muscles very carefully until the sciatic nerve is exposed.   Dissect out the nerve as long as possible, and moisten frequently with the normal salt solution.   Remove all of the muscles as far as the knee, leaving the femur and nerve entirely isolated.   Avoid all injury to the nerve during dissection, and apply the normal salt solution every few minutes with a camel's hair brush.   Arrange the nerve muscle preparation by placing the femur in a clamp and allowing the nerve to hang freely.   A small lever may be pinned to the foot to emphasize any movements that may occur.   Apply the following stimuli :

320.   *Mechanical.*   Pinch the free end of the nerve sharply with a pair of forceps ; the muscles contract and the foot is raised suddenly.   Cut off the pinched portion.   Contraction again occurs.

321.   *Thermal.*   To the same preparation apply at the free end of the nerve a wire or needle heated to a dull heat or a lighted match.   Contraction again occurs.   Cut off the dead part of the nerve.

322.   *Chemical.*   Place some saturated solution of sodium chloride in a watch glass and let the free end of the nerve dip in it.   It requires a few moments for the salt to diffuse into the nerve, on account of the difference in the specific gravity.   Soon the joints of the toes twitch and by-and-by the whole limb is thrown into irregular, flickering spasms, which terminate in a more or less continuous contraction, constituting *tetanus.*   Cut off the part of the nerve affected by the salt ; the spasms cease.

(a)   Finish the experiment by exposing the nerve to the vapor of strong ammonia in a test tube or bottle.   The ammonia must not act directly upon the muscle, the tube should be raised slightly above the level of the muscle and the end

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of the nerve elevated to the mouth of the tube. There should
be no contraction if the vapor has not come in contact with
the muscle. The ammonia kills the nerve. Apply ammonia
to the muscle. It contracts.

323. *Electrical.* For the following experiments, use the
other leg of the frog, taking the same precautions in the dis-
section and application of the normal salt solution.

(a) Arrange the nerve-muscle preparation with the fe-
mur in a clamp and connect with a recording lever. Arrange
the battery and DuBois Reymond Key with the induction coil.
Connect the battery wires with the primary coil and remove
the secondary coil to the lower end of the scale. Arrange the
electrodes with a short-circuiting key and a recording drum
with smoked paper, conveniently to the preparation. When
all is in readiness, close the connection by means of the key
by raising or lowering its lever. Or in other words " making "
or " breaking " the current. Gradually move the secondary
coil along the scale while making and breaking the current.
The current is made when the connection is complete, and
broken when the connection is interrupted. Note at what
point on the scale the first result appears and whether it be
from " make " or " break." Make a table of your results as
follows : In one column indicate the distance of the second-
ary coil from the primary. In another, Response at Make,
and the last column, Response at Break. This is electrical
stimulation in the form of *single induction shocks.* The highest
tracings represent maximal and the lowest minimal stimuli.
Submaximal stimuli represent any strength between these
two extremes.

(b) Remove the induction coil and use only the battery
with its wires and the key. Make and break the current as
in (a). Notice that if the key be so arranged as to permit
the current to flow continuously through the nerve, no con-
traction occurs, provided there be no variation in the intensity
of the current. Rapidly make and break the current by open-
ing and closing the key ; a more or less perfect tetanus is pro-
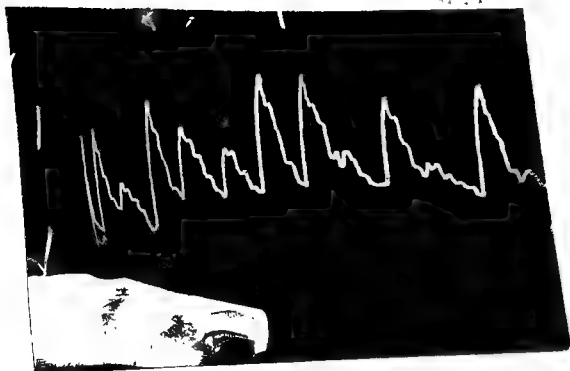duced. This is the *constant current* form of stimulation.

(c) Remove the wires from the primary coil and connect
them with the sockets leading to the vibrating hammer. On
applying the electrodes to the nerve or muscle the latter is at

Breath

Ictin

M + B
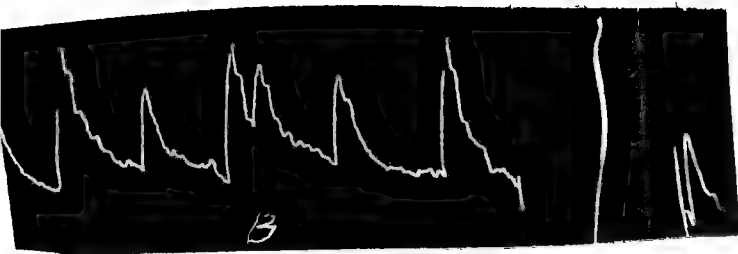
once thrown into a state of rigid spasm or continuous con-traction called tetanus. Compare this tracing with that of (a). This form of stimulation is known as the *interrupted current* or repeated shocks.

324   Electricity itself is not readily conveyed through the nerve, but the irritation caused by it, generates a stimulus which is transmitted, Ligate the nerve by tying tightly around it a piece of thread.   Stimulate the nerve as before ; there should be no result, as the ligature has crushed the nerve and blocks the passage of the stimulus. Scratch your name on the above tracings to identify them. The tracings may be made permanent by drawing the paper through a pan of shellac.

XXIII

325. SECONDARY CONTRACTION. Arrange the induction apparatus for single make and break shocks. Pith a frog and use the hind legs for nerve-muscle preparations, and ar-range them upon a glass plate. Place the sciatic nerve of the left leg upon the gastrocnemius muscle of the right leg, fig. 20. Place the sciatic nerve of the right leg over the elec-trodes and stimulate the nerve with single induction shocks
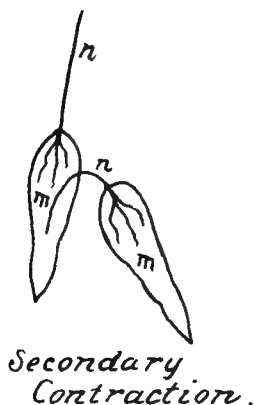

Secondary Contraction.

FIG. 20.
Fig. 20, *n*, nerve ; *m*, muscle.

and note that the muscles of both the right and left leg contract. The contraction in the left leg is called a secondary contraction. Repeat the experiment, using the constant current. Note if there is any difference between the make and break shocks.

326. SECONDARY TETANUS. Prepare the induction apparatus for interrupted shocks, and again stimulate the right sciatic nerve. The right gastrocnemius muscle is thrown into tetanus. The left gastrocnemius is simultaneously tetanized. This is known as a secondary tetanus, and is a proof of the " action current " in muscle. The left sciatic is stimulated by the variation of the muscle current during the contraction of the right gastrocnemius. Ligate the left sciatic near its muscle ; stimulate the right sciatic ; there should be no contraction of the left gastrocnemius.

Leaving the left sciatic in position, tie a ligature around the right sciatic near its muscle and stimulate. Is there contraction in either preparation ?

This experiment also shows that electricity, as such, is not transmitted through the nerve as the thread of the ligature is a conductor. The electricity serves merely as a stimulus causing an impulse which can traverse the normal nerve but cannot pass beyond a ligature or a crushed portion of the nerve.
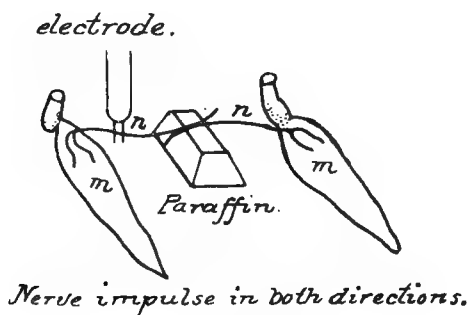

Fig. 21

Fig. 21, *n*, nerve ; *m*, muscle.

327. SECONDARY CONTRACTION FROM NERVE. Make a nerve-muscle preparation of the right hind leg of the frog and

lay it on a glass plate. Dissect out a long piece of the left sciatic nerve. Remove and arrange it in such a way upon a block of paraffin that one centimeter of it overlaps a corresponding length of the right sciatic, fig. 21.

Stimulate the left nerve with a single induction shock; the muscle contracts. Stimulate with the interrupted current; the muscle is thrown into tetanus. Stimulate also with the constant current and compare effects. If properly conducted this experiment will also show that a nerve impulse can pass in both directions.

Stimulate the left muscle directly by applying the electrode to it and note any effect upon the right muscle.

Ligate the left sciatic nerve between the electrodes and the right nerve; stimulate again. The muscle does not contract. In the former case, therefore, its contraction was not due to an escape of the stimulating current.

328. SECONDARY CONTRACTION FROM THE HEART. Excise the heart; lay the nerve of a fresh nerve-muscle preparation upon it as per diagram, fig. 22. The muscle contracts at each beat of the heart, being excited by the electrical current which accompanies each beat.

FIG. 22.

FIG. 22.   Secondary contraction from the heart.

Crush the apex of the ventricle with the forceps and arrange the nerve so that its cut end will come in contact with the injured portion of the heart. Note the result.

329. PARADOXICAL CONTRACTION. Arrange the battery and key for a constant current.

Pith a frog and expose the sciatic nerve down to the knee. Trace out the two branches into which it divides, fig. 23. Cut off one of these branches as near as possible to the knee and stimulate near its cut end. The muscles, supplied by the other branch of the nerve, contract. Try mechanical or chemical stimulation of the same branch. What is the result?
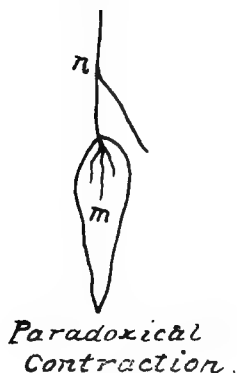
n

m

Paradoxical Contraction.

FIG. 23.

FIG. 23, *n*, nerve ; *m*, muscle.

The second branch is stimulated by the electrotonic alteration of the first.

Electrotonus—modification of the vital properties of irritable and contractile tissues when influenced by a constant battery current. (Stirling).

330. EXPERIMENT WITH ERGOGRAPH. Adjust the apparatus so that the lever will write upon a drum revolving at its lowest rate of speed. Tie the three fingers of the right hand leaving the index finger free. Adjust this finger to the vertical rod connected with the lever. Raise and lower the finger thus causing a simultaneous movement of the lever, which leaves its record upon the smoked paper. Raise and lower the finger at regular intervals (one second). The *abductor indicis* is the principal muscle involved. Continue until distinct fatigue occurs. Fix and preserve the tracing.

XXIV

331. ELASTICITY AND EXTENSIBILITY OF MUSCLE. Dissect out the gastrocnemius muscle from the frog's leg. Attach the femur firmly in the muscle clamp and the tendon to the writing lever, to which a small scale pan is attached. See that the lever writes horizontally on a stationary drum. The weight of the scale pan may be neglected.

Place in the scale pan, successively, different weights ; 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 or more. Put in the 10 gram weight. The lever will descend. Remove the weight and the muscle will return to its original position. Replace the weight, the lever will drop along the line it first made, revolve the drum a very short distance horizontally and add 10 grams more ; the lever will descend. Revolve the drum again for a short distance equal to the previous horizontal distance, add 10 grams more and repeat the above processes until the heavy weights have been used. The " steps " of the " staircase " will become shorter and if the apices of all of the " steps " be joined, the line will form a hyperbola. At the end of the experiment remove the weights and note any contractile phenomena. Does the muscle return to its original position ?

Repeat the experiment with a thin strip of rubber, substituted for the muscle. Join the apices of the " steps " with a line and compare with that of the preceding experiment.

Test in the same way the elasticity of a short strip of aorta (cat) provided for the experiment.

Test also a short piece of the frog's intestine.

332. INDEPENDENT IRRITABILITY OF MUSCLE. Apparatus : Battery, induction coil, two keys, wires, electrodes, curare, etc.

Arrange the battery and induction coil for an interrupted current with one key in the primary circuit and the other key to short-circuit the secondary.

Destroy the brain of a frog. Expose the sciatic nerve and the accompanying artery and vein, on the left side, being *very* careful not to injure the blood vessels. Isolate the sciatic nerve and tie a stout ligature around all of the other structures of the leg.

Inject 5 to 10 drops of the curare solution into the abdominal cavity. The poison will be carried to every other part of the body except the leg below the left ligature. The animal is paralyzed in from twenty to thirty minutes. If the non-poisoned left leg is pinched it is drawn up, showing that it has not lost its reflex powers ; while the poisoned right leg has lost its reflex.

When the frog is thoroughly under the influence of the
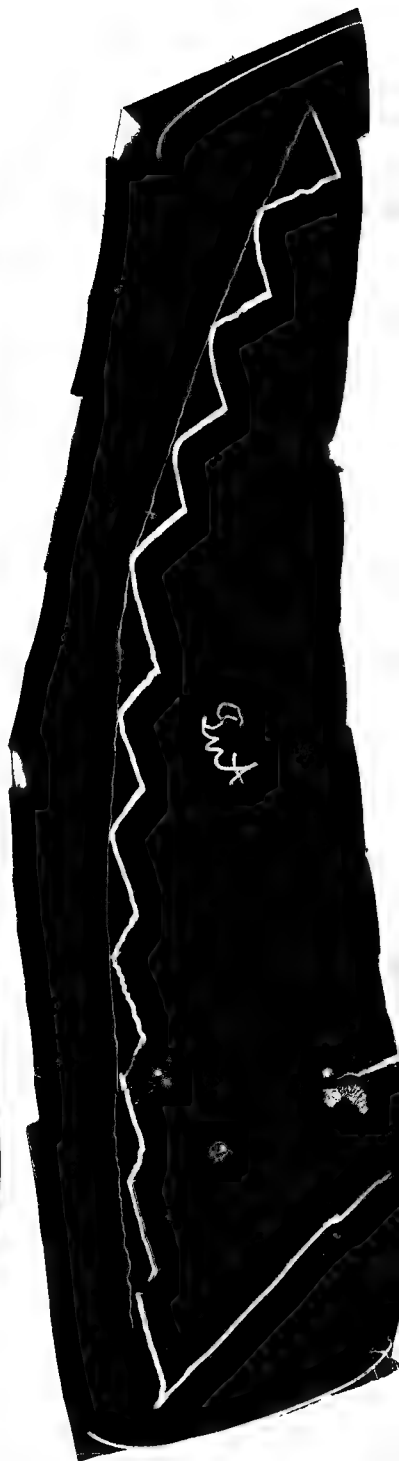
poison, *i. e.*, when all reflexes cease, expose both sciatic nerves as far up as the vertebral column and as far down as the knee.

Stimulate the right sciatic nerve. There is no contraction. Stimulate the right gastrocnemius muscle ; it contracts. The poison has therefore not affected the muscle.

Stimulate the left sciatic nerve above the ligature, the left leg contracts. The poison has not affected the nerve trunk. The nerve impulse is blocked by the curare, in all probability by paralysis of the end plates of the motor nerves within the muscle. Apply several drops of a strong solution of curare to the left gastrocnemius, and after a time stimulate the left sciatic nerve ; there should be no contraction, but on stimulating the muscle directly, contraction occurs.

333. BERNARD'S METHOD. Two nerve-muscle preparations are made. The nerve of one (A) is immersed 20 to 30 minutes in a solution of curare in a watch-glass. The muscle of the other preparation (B) is immersed in the curare in another watch-glass for an equal length of time. On stimulating the nerve of A, its muscle contracts ; on stimulating the nerve of B, its muscle does not contract, but the muscle contracts when it is stimulated directly. In A, although the poison is applied directly to the nerve trunk, the nerve is not paralyzed.

334. RELATIVE EXCITABILITY OF MUSCLE AND OF NERVE. Determine the minimal break shock which will cause a muscle twitch through the sciatic nerve, and then apply the same stimulus to the gastrocnemius muscle directly. It will not cause contraction.

Slide the S coil nearer to the P coil until the stimulus is strong enough to cause the muscle to contract, and note the difference in strength required.

This experiment does not permit of the conclusion that the muscle possesses independent irritability, as the nerve terminations in the muscle are not excluded. (See Curare Experiment.)

335. CHANGES IN THE EXCITABILITY OF A NERVE WHEN DYING. Dissect out the sciatic nerve of a frog, but do not cut it from its connection with the myel. Place under its whole length a strip of thin rubber or a piece of waxed paper and keep the nerve moist with the saline solution.

Carefully raise the nerve with the glass rod or camel's hair brush and explore it from one end to the other with minimal single induction break shocks, the effect of which have been tested first at the middle of the nerve, and note if

No contraction occured.

there is a difference in excitability at any point. There is usually one or two such points. Locate them.

Determine this by the change produced in the muscular effect, such as an increase, decrease or absence of contraction.

Now cut the nerve at its spinal origin, and compare the excitability at the cut end with that at a point near the muscle. Repeat this from time to time. The cut end will soon show a greater excitability which will decrease later until it is completely lost.

A dying nerve first rises and then falls in excitability and finally loses it altogether. A nerve undergoing the process of drying becomes for a time, more irritable for this reason.

336. DEAD MUSCLE AND NERVE. Immerse a nerve-muscle preparation for a few minutes in warm water (40 degrees C). Apply all of the preceding stimuli and compare results with those obtained from a normal preparation.

337. KUEHNE'S SARTORIUS EXPERIMENT. Carefully dissect out the *sartorius* muscle, and to the tendon which attaches it to the tibia tie a fine thread. The upper end of the muscle may be freed from its attachment to the symphysis. Suspend the muscle with its upper end hanging downward and bring up under it a little glycerin in a watch-glass until the end of the muscle just touches the glycerin. Observe for a minute or two. No contraction should result. Cut off the end which has touched the glycerin and note that the muscle contracts as a result of the mechanical excitation. Again touch the cut surface with glycerine and observe. If only about one millimeter has been cut off there is again no contraction. Cut off a fresh millimeter of muscle and repeat as before. It will be found that when about three or four millimeters of the cephalic end have been cut away, on contact of the freshly exposed end with the glycerin, the muscle shows irregular twitchings and is at last thrown into a state of incomplete tetanus.

This experiment should be completed by showing that if a gastrocnemius nerve-muscle preparation be made and the cut end of the nerve dipped into glycerine, the gastrocnemius is thrown into a similar series of irregular twitching. Nerve fiber is therefore excitable to glycerin. The same experiment may be tried upon a curarized muscle.

The experiment on the *sartorius* muscle confirms the fact, as shown by histological examination, that no nerve fibers are present in the ends of the muscle ; for the same experiment shows the same results for two or three millimeters of the distal end of the muscle. Muscle fiber is not excited by glycerin and not until enough of the muscle was cut away to expose the nerve fibers in the muscle did the irregular twitchings occur.

XXV

338. THE MOIST CHAMBER. Muscle and nerve tissue dry shortly after their removal from the body and the usefulness of an experiment is, sometimes, much hampered by this fact. In order to prevent drying, a moist chamber is employed. This apparatus consists of a glass cover fitting tightly over the myograph (muscle electrodes). A thread passing through an aperture connects the muscle with the recording lever. A few pieces of blotting paper wet with the normal saline solution placed in the chamber keeps the air moist.

339. THE MUSCLE CURVE. If a stimulus of very short duration be applied to a muscle or its nerve, the muscle responds by giving a contraction of very short duration. This is termed a simple twitch.

The curve obtained from a muscle falls naturally into three parts :

(1). From the point of stimulation (a) to the point of commencing contraction (b). This is known as the Latent Period. During this time there is no change in the length of the muscle. A muscle does not contract simultaneously all over, but the contraction starts at some one spot and then spreads in a wave-like manner over the rest of the muscle. Following an excitation at one spot, the fibers in that position contract, but do not at first lead to a movement of the recording lever but rather to a stretching of the remainder of the fiber, both above and below the point of contracting. The parts which have to be moved possess some inertia.

(2). From the point of commencing contraction (b) to the highest point of the curve (c). This is termed the Period
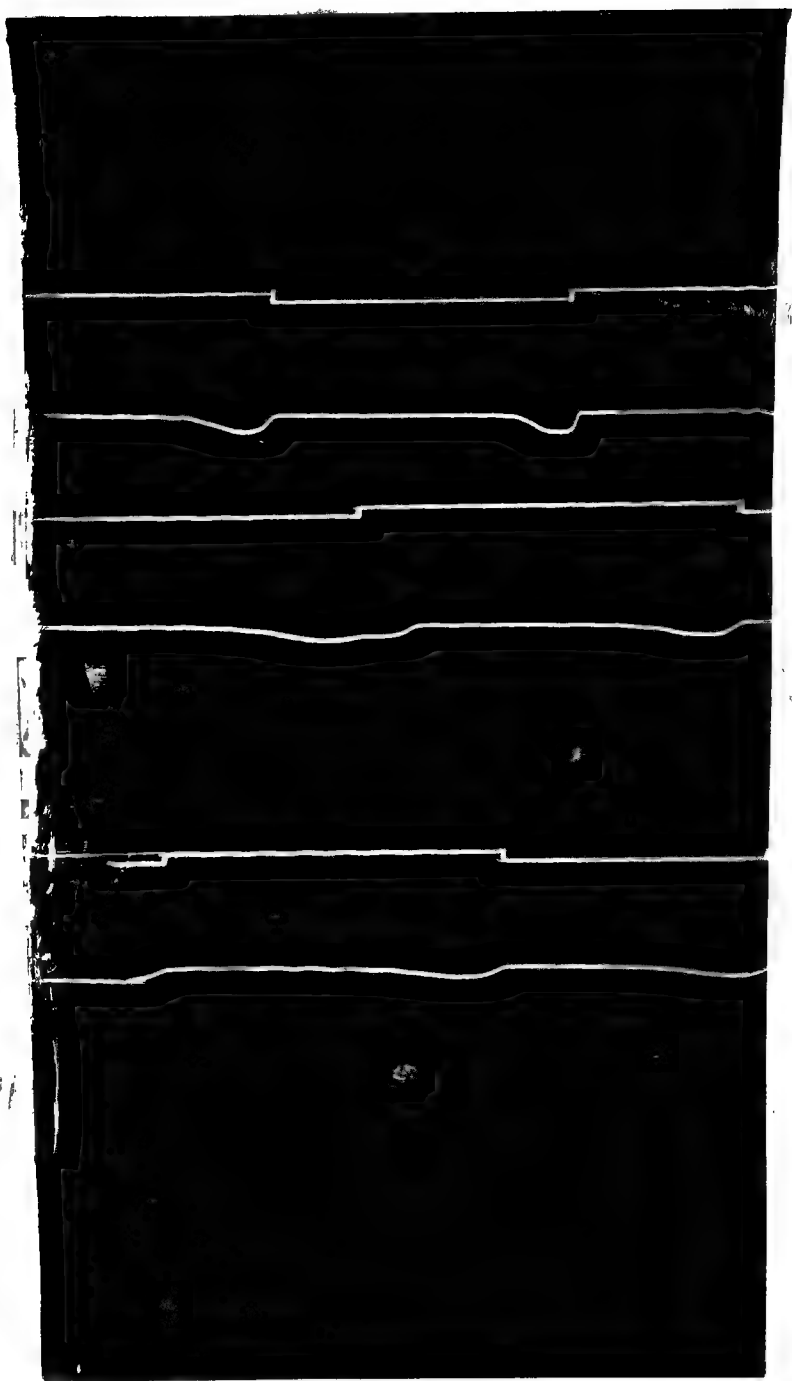
of Contraction. The curve is at first convex to the abscissa, or base line, which means that the rate of contraction is at first very slow as seen by the acute angle which the first part of the curve makes with the abscissa ; it then rapidly increases as shown by the increasing inclination to the abscissa, and very soon reaches a maximum rapidity. From this, again, there is another change in rate, this time in the reverse direction, for the curve becomes concave to the abscissa line and gradually shortening becomes slower until at last it ceases, when the tangent to the curve becomes parallel to the abscissa.
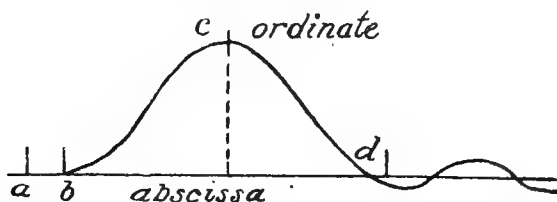

FIG. 24

Fig. 24, *ab*, latent period ; *bc*, period of contraction ; *cd*, period of relax-ation.

(3). The third portion of the tracing is from the highest point (c) to the point (d) at which the lever again reaches the abscissa. This part is called the Period of Relaxation. The terminal point (d) is often a difficult one to determine with any accuracy because the lever does not come instantly to rest ; but as it always possesses some inertia, it oscillates for a time about a mean position which it ultimately reaches.

340. Arrange a nerve-muscle preparation in the moist chamber so as to record its contraction upon the drum. Con-nect the battery with the induction coil introducing into the primary circuit a make and break key and an electro-magnet. Use also a short-circuiting key in the secondary circuit. Ar-range the writing tip of the lever from the electro-magnet, so that it will write just below and on the same vertical plane as the muscle lever. Spin the drum at a fairly rapid rate by hand and use single induction shocks by breaking the primary circuit. The lever of the electro-magnet will indicate the instant the current is sent into the nerve-muscle preparation. (The lever rises and falls alternately as the current is made

or broken). The muscle lever will rise just after that of the magnet. The tips of the two levers having started side by side from the same vertical plane, the difference on the two abscissas between the rising point of the lever from the magnet and the rising point of the muscle lever, will be the approximate latent period. Verticals drawn through the two abscissas at the rising points of the two levers will be of use in determining more accurately the extent of the latent period.

Verify as far as possible on the tracing, the preceding statements. Also obtain a curve from the make shock alone (short circuit the secondary coil when the break shock should occur). Vary the position of the secondary coil and compare the curves. Get a tracing of the contraction of plain muscle, by using a piece of the intestine or stomach of the frog. Compare.

341. AMPLIFICATION OR MAGNIFICATION. The amplitude of the tracing is measured by the ordinates; it is the distance which separates each point of the tracing from the line of the abscissa. When the primitive length of the muscle does not change as in the period of latent excitation, this distance equals 0, and the tracing is confounded with the line of the abscissa: When the muscle shortens, the tracing is raised above this line to a height relative to the degree of shortening. When the muscle elongates the tracing falls below this line to a certain extent. But as the muscle acts on a long lever, the changes in the length of the muscle are amplified on the tracing in a noticeable way. If, for example, the lever has a total length of 150 millimeters and the tendon of the muscle is attached to a point 15 millimeters on the axis of rotation of the lever; each millimeter of muscle shortening will be produced on the tracing by a height (amplitude) of ten millimeters (1 centimeter).

It is not difficult when one knows the length of the lever and the distance from the point of attachment to the axis, to calculate the actual degree of muscular shortening.

Amplitude depends upon the length of muscular fiber; the longer the fibers of the muscle the longer the curve of amplitude. In general the amplitude increases with the intensity of the excitation, but there is a limit.

Determine the amplitude in your tracing by measuring

the length of the lever in millimeters ; then measure from the point of attachment of the muscle to the fulcrum and divide the total length by this and the result will give the degree of magnification of the shortening of the muscle.

342. WORK DONE DURING A SINGLE CONTRACTION. Arrange a gastrocnemius to record on a cylinder, but record only the "lift," the cylinder being stationary, move the cylinder by the hand as required. On the lever under the muscle attachment place weights of known value. With each twitch the muscle lifts the weight, and thus does a certain amount of work which is easily calculated.

(a). Measure the height of the tracing from the base line or abscissa. This is conveniently done by a millimeter scale. The work which is done (w) is equal to the load (1) multiplied by the height (h) to which it is lifted ; w=lh. But, of course, a long lever being used the tracing is much higher than the actual shortening of the muscle.

(b). To determine the exact amount of the lift, one must know the length of the lever and the ratio between its arms. Suppose the one to be ten times as much as the other, then the total work in gram-millimeters must be divided by ten.

Try different weights always using the same stimulus. It will be found that at a certain point there will be a maximum contraction after which the contractions will become weaker, because of the greater load and fatigue. Calculate the amount of work done at the maximum contraction.

343. RECORD OF THE THICKENING OF A MUSCLE. Prepare a nerve-muscle preparation and lay it on a glass plate, keeping it well moistened with the saline solution. Arrange the battery and induction coil as before. Adjust the heart lever (such as used in recording the beat of the frog's heart) so that the vertical portion of the lever rests upon the belly of the muscle. Use the break and then the make shocks as before. Compare these curves with the others. The drum should revolve at its fastest rate.

ad 5·0 gr   1 cm = 5 cm
                        20 cm 1:4
H 40 cm

. 4 (2000 W.
       ‾‾‾‾‾‾
       5·0 gm

/ 2 m − 3 =   ratio 1:4

        H = 3 mm
        wt = 20 gr
                ‾‾‾‾
        W ·· 60 ÷ 4 = 15 W actual.

XXVI

344. INFLUENCE OF VERATRINE ON THE CONTRACTION OF MUSCLE. Destroy the brain of a frog, and inject hypodermically four or five drops of a 1% solution of sulphate of Veratria. When the frog is under the influence of the poison, cause a reflex act by mechanically stimulating the skin of the leg. The limbs are extended, and remain so for several seconds, due to the prolonged contraction of the extensors overcoming the flexors and thus causing extension of the legs.

Arrange the induction machine for single shocks and make and break the primary circuit by means of the key. Short-circuit the secondary. Do not stimulate the muscle often as the veratria effect diminishes with the activity of the muscles.

Make a nerve-muscle preparation, on cutting the nerve notice the prolonged extension of the legs.

Arrange the muscle lever to record its movements on a slow moving drum. Take a tracing. Note that the muscle contracts quickly enough, but the contraction is very high compared with that of a non-poisoned muscle, while the muscle relaxes very slowly indeed. The relaxation phase may last several seconds. The tracing may show an uneven curve due to irregular spasms of the muscular fibers.

Take another tracing with a quick revolving drum, and a curve reaching the whole circumference of the drum may be obtained, or the drum may go around several times before the relaxation is complete.

Note that if the " veratrized " muscle be made to contract several times the effect passes off—only a simple twitch being obtained—but is re-established after rest. A high temperature also causes it to disappear.

345. FATIGUE OF MUSCLE. Arrange an induction coil for break shocks, i. e., adjust the strength of the stimulus so that only one, the break and not the make, will appear. Prepare a nerve-muscle preparation, use a long lever and a weight of 50 grams. Use a slow revolving drum on which to record the muscle tracings, so slow that the ascent and descent of the lever form merely one line. Break the primary current at regular intervals.
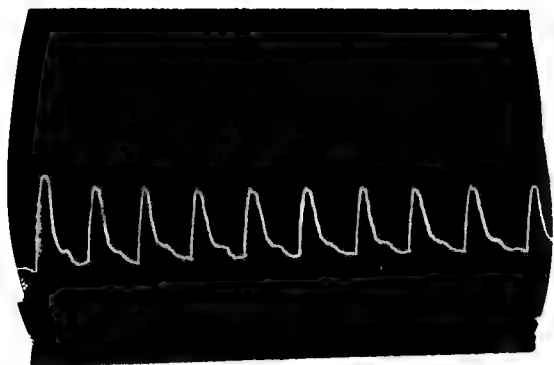
Note the "staircase" character of the record, *i. e.*, the second contraction is higher than the first, the third than the second and so on for a certain number of contractions. After that the height of the contraction falls steadily so that a line uniting the apices of all of the contractions forms a straight line approximately. Note later that in the phase of relaxation the lever does not reach the abscissa. If the march of events be arrested, and time given for repose, then, on stimulating, the lift increases, but the effect lasts only for a short time.

After the gastrocnemius muscle is thoroughly fatigued cut across the middle of the muscle with a scalpel and test with litmus paper the area of the cross section thus exposed. Test in the same way a cross section of the *sartorius* or some other muscle which has not been fatigued.

346. TETANUS. Prepare a nerve-muscle preparation. Arrange to record on drum with the smallest fan.

Place a key in the primary circuit, also one in the secondary and wire for interrupted current. Adjust the special wire and weight in the vibrating hammer, so that it swings at its lowest and gentlest rate. Open the short-circuiting key in the secondary circuit. Make the current for very short intervals in the primary. Adjust the weight so as to get faster vibrations and compare.
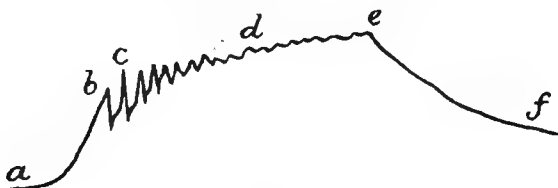
FIG. 25.

Fig. 25. Curve of tetanus. At the beginning *ad*, the individual contractions are somewhat discernible ; these disappear and the general level of the curve rises to *e*. At this point the stimulus was removed and the curve dropped quickly toward the base line.

Study the tracings. The first are indented but gradually there is more and more fusion of the teeth until a curve unbroken by depressions is obtained. In the curve of complete tetanus the ascent is at first steep then slightly more gradual, speedily reaching a maximum, when the lever practically

records a horizontal line parallel to the abscissa. When the current is shut off the descent is very steep at first, and towards the end very slow.

The number of shocks required to produce tetanus depends on the animal, the muscle, and the condition of the latter ; the more fatigued a muscle is the more slowly it contracts, and therefore, the more readily does fusion of contractions take place. A fresh frog's gastrocnemius requires about 27 to 39 shocks per second to produce tetanus.

Replace the key in the primary circuit by a metronome ; connect the wires with the primary coil. Vary the rate of vibration of the metronome and observe the effect on the muscle curve. Compare with the previous tracings.

XXVII

347. INFLUENCE OF TEMPERATURE UPON THE CONTRACTION OF MUSCLE. Prepare a gastrocnemius muscle, leaving it attached to the femur. The sciatic nerve may be disregarded. Fasten the femur in the clamp on the under side of the cover of the " muscle warmer." Tie the end of a fine copper wire around the tendon of Achilles. Bring the other end of the wire through the. opening in the bottom of the muscle warmer and bend the wire around the muscle lever, making sure that the wire connecting the tendon with the muscle lever is vertical. Connect the end of the fine copper wire with one of the binding posts of the secondary coil. Connect the other·post of this coil with the binding post on top of the cover of the muscle warmer. Connect the battery with a make and break key to the binding posts of the primary coil of the inductorium for single induced shocks. Fill the outer chamber of the muscle warmer with crushed ice. Bring the writing point of the muscle lever againt a smoked drum and let the drum revolve at a fairly rapid speed. Insert a thermometer in the top of the muscle warmer and stimulate the cooling muscle at intervals of 5 degrees with a maximal break current. As the temperature falls, the contraction curve becomes longer and the muscle shows a tendency to contracture. Indicate the temperature upon each curve made.

Place a fresh paper on the drum and let it revolve slowly. Adjust a flame under the arm of the muscle warmer and stimulate the muscle with a maximal break current at intervals of 5°. Indicate the temperature upon the curves as before.

"The height of contraction is least at the freezing point of the muscle (—5 degrees). It rises from the freezing point to zero ; falls from zero to 19 degrees ; increases to 30 degrees, which is the maximum ; from 30 to 45 degrees diminishes again, and at 45 degrees the frog's muscle usually enters into a state called rigor caloris ; the muscle becomes opaque, inelastic, resistant to the touch, shortens very considerably and undergoes chemical changes of great importance. The duration of contraction lessens with the rising temperature, being least at 30 degrees. Above 30 degrees the duration remains practically unchanged. The latent period is increased at low temperatures, diminished at high. Above 30 degrees the excitability to electrical stimuli diminishes steadily ; it disappears almost entirely before rigor is reached."—Porter.

348.—THE INFLUENCE OF LOAD UPON THE CONTRACTION OF MUSCLE. A muscle to which a load is suspended is said to be " loaded." If a muscle contracts against a small and constant resistance, so as to be extended by a constant force during its contraction, the curve described by a light lever attached to it is termed "*isotonic.*" If a muscle contracts against a large resistance, *e. g.*, a strong spring, so that it can shorten very little, the curve described by a lever attached to it is termed "*isometric.*" The latter is flat-topped, *i. e.*, it shows a period of maintenance at maximum contraction. The muscle reaches its maximum tension sooner than its maximum shortening and maintains the maximum tension longer than the maximum shortening. As ordinarily employed, the myograph gives an isotonic curve.

Arrange the apparatus as for a simple twitch. Arrange a nerve-muscle preparation and attach to a lever weighted only with the scale pan. Obtain a tracing as thus arranged. Increase the load by adding a 10 gram weight and get tracing. Increase the load still further by adding 30, 50 and 100 grams respectively and compare the tracings.

It will be noticed that the latent period increases as the load increases ; the period of contraction also tends to increase ; the period of relaxation is at first decreased but with heavier loads is increased ; the heights of the contraction diminish progressively as the load increases.

349. AFTER LOAD. In the ordinary study of muscle twitch, the contractions have occurred whilst the load on the muscle was as nearly as possible constant. There is, however, a method which is exemplified in many bodily movements, in which the muscle is under a low tension until it commences to contract, and then, only, experiences a rise of tension. This is called the method of after loading. Arrange the apparatus for taking a simple twitch with a nerve-muscle preparation. Load the muscle with a weight of 20 grams attached to the pully of the muscle lever. Have the screw of the muscle lever adjusted so that the muscle itself bears no weight. Now lower the screw so that the whole load is carried by the muscle and bring the writing tip of the lever so that it will write horizontally upon the drum. Get a tracing of the muscle curve, using the break shock only. Now raise the screw to support the writing lever, so that the writing point is placed at the level of the apex of the curve just taken. Record from this position another curve. It will be found that the muscle still raises the lever. Raise the screw again until the level of the writing point is at the summit of this second curve, and again get tracing. Repeat this process until no further result is obtained.

The important and characteristic feature of these curves is that though the weight is supported at a level which it just reached at the height of a previous contraction, it is still further raised when the muscle is again stimulated. Under such conditions, the muscle contracts to a greater degree than when freely loaded. The latent period is increased as the height of support of the weight is increased. This is accounted for by the fact that the muscle is taking in any " slack " there may be ; also that it is gradually increasing its tension until it is able to lift the load. The first portion of such a twitch is isometric ; but beyond a certain point it suddenly becomes isotonic, and its shortening is then registered.

350. INDUCTION IN NERVES. Remove the secondary coil and with the aid of a glass plate, lay the nerve of a well moistened nerve-muscle preparation upon the primary coil in such a way that the free end of the nerve touches the nerve near the muscle or touches the muscle itself, so as to form a closed circuit. Make and break the primary circuit. Make and break currents will be induced in the nerve and the muscle will contract.

351. TELEPHONE EXPERIMENT. Arrange a nerve-muscle preparation with its nerve over a pair of electrodes. Connect the latter with a short-circuiting key. Attach the key to the telephone by means of two wires. Open the short-circuiting key ; shout or blow into the telephone, and note that the muscle contracts vigorously. Remove the electrodes from the key and connect directly with the telephone. What is the result ?

XXVIII

352. CARDIC VAGUS OF THE FROG. Refer to the previous dissection of the vagus. Pith the frog. Lay it on its back on a frog board. Expose the heart, remove the sternum and pull the fore legs well apart. Distend the oesophagus by introducing a glass rod or tube ; the nerves leaving the cranium are better seen winding from behind when the oesophagus is distended. Remove such muscles as may be necessary. Three nerves are seen coursing round the pharynx. The lowest is the hypoglossal, easily recognized by tracing it forward to the tongue, next is the vagus in close relationship with a blood vessel, and finally the glossopharyngeal. Observe the laryngeal branch of the vagus. The vagus as here exposed, outside the cranium, is really the vago-sympathetic as it contains fibers from the sympathetic system. The glosso-pharyngeal and vagus leave the cranium through the same foramen, in the exoccipital bone, and through the same foramen the sympathetic enters the skull.

Stimulation of the vagus. Adjust a heart lever so as to record the contractions of the heart upon a drum moving at a medium rate of speed. Place well-insulated electrodes under

350 Muscle Contracts.
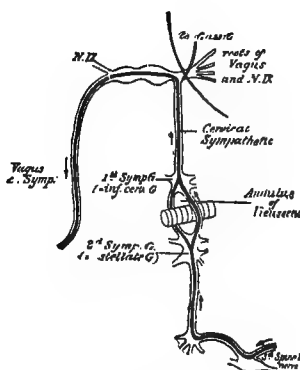
351 Demonstrated by instructor

FIG. 26

Fig. 26.—Cardiac Nerves of the Frog.   (Foster).

the trunk of the vagus, stimulate it with an interrupted cur-
rent, and observe that the whole heart is arrested in diastole.

If the stimulation is kept up the heart will finally "escape"
from the influence of the stimulus and will recommence beat-
ing.   Note if the auricles appear to be more inhibited than
the ventricle.

There is often a difference in effect between the two vagi.
Sometimes one vagus is found not to possess any inhibitory
fibers, in which case the opposite vagus is usually found
especially active.   It is generally found that the effect is not
identical on the two sides, one usually being more powerful
than the other.

The arrest, or *period of inhibition*, is manifest in the curve
by the lever recording merely a straight line.   If the laryn-
geal muscles contract, and thereby affect the position of the
heart, divide the laryngeal branches of the vagus.

There is an appreciable time or latent period, before the
heart reacts to the stimulus and likewise when the stimulus
is removed the heart does not at once regain its normal move-
ment.   .Note that when the heart begins to beat again the
beats are at first small and gradually rise to normal.   In some
instances, however, they are more vigorous and quicker.   Cut
both vagus nerves and compare the tracing with those just
obtained.

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353. ACTION OF DRUGS ON HEART. The experiments may be performed upon a heart which has been removed and placed in a watch-glass or preferably upon the heart in its natural position.

Muscarine. Pith a frog, expose its heart and with a fine pipette apply a drop of serum or normal saline solution containing a trace of muscarine, which rapidly arrests the rhythmical action of the heart, the ventricle being relaxed, *i. e.*, diastole, and distended with blood. Get a tracing from the heart while under the influence of muscarine.

Atropine. Remove the solution of muscarine as much as possible by absorbing it with filter paper and after a few minutes, with another pipette, apply a few drops of a 0.5% solution of atropine sulphate in normal saline, the heart gradually again begins to beat rhythmically. Get a tracing.

Pilocarpine. In another frog arrest the action of the heart by applying a few drops of a 0.5% solution of pilocarpine, and then apply atropine to antagonize it, and observe that the heart beats again after the action of the atropine.

Nicotine. Apply a drop or two of a 0.2% solution of nicotine. Stimulate the vagus and note that it no longer arrests the heart's action, but stimulation of the sinus venosus does ; so that nicotine paralyzes the fibers of the vagus and leaves the intracardiac inhibitory mechanism intact.

XXIX

In this exercise four styles of sphygmographs are available. Each student should obtain a tracing of his pulse from these instruments ; fix and preserve the records for comparison.

354. LUDWIG'S SPHYGMOGRAPH. With a soft pencil make a mark upon the skin of the wrist at the point where the radial pulse is most distinctly felt. Apply the instrument so that the button rests upon the pulse. Use the arm rest so that the parts will remain steady. Adjust the instrument so that the writing lever will move freely and give as large a curve as possible. Take tracings on the revolving drum. Suspend respiration for a few seconds and notice whether there is any effect upon the pulse.

355. VON FREY'S SPHYGMOGRAPH. Adjust in the same manner as for Ludwig's. See that .ne clockwork runs properly and take tracing on the small drum.

356. RICHARDSON'S SPHYGMOGRAPH. Adjust the pressure of the button upon the artery until a maximum excursion of the marker is obtained. Wind up the clock-work, insert a strip of smoked paper between the guide wheels, and let the paper travel past the recording point as soon as the latter moves regularly.

357. TESKE'S SPHYGMOGRAPH. Apply this instrument to the radial artery so that the amplitude of the pulse is at its greatest. When all is in readiness, set the smoked paper in motion by pressing the lever. Care must be taken not to move the instrument nor the arm of the person while the pulse is being registered.

This instrument magnifies the movements of the artery fifty times. The clockwork is regulated so that the smoked paper shall pass through in ten seconds. Six times the number of pulsations traced on the paper will give the number per minute. The clockwork will not pass more than two lengths of the paper at the same rate. It is best to wind it up anew after two lengths have passed.

358. SPHYGMOMETER. (Von Basch). Find the radial artery where it is most superficial and apply two or three fingers along its course. Note that the pulse can be distinctly felt by all three fingers. Compress firmly with the middle finger. What can you detect with the other two fingers? Compress with the first finger. What results are noted by the other two?

Place the ampulla of the sphygmometer upon the artery at the point of most distinct pulsation. Press gradually but steadily until a point is reached on the scale where the needle oscillates at every beat of the pulse. The pressure will then be the same inside and outside the vessel. This measures the blood pressure. The figures on the scale of the apparatus indicate centimeters of mercury.
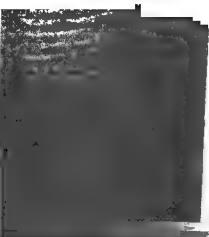
Count your pulse for a full minute, in the standing and sitting positions. Also keep a record of the pulse during different periods of the day, e. g., morning, noon and night.
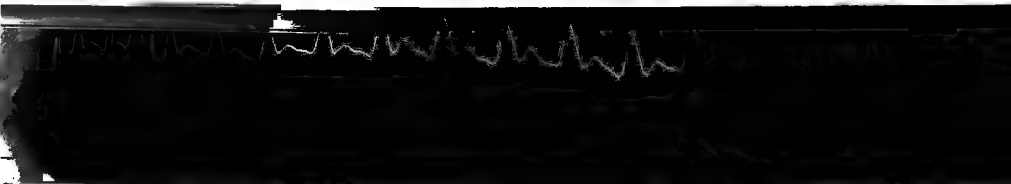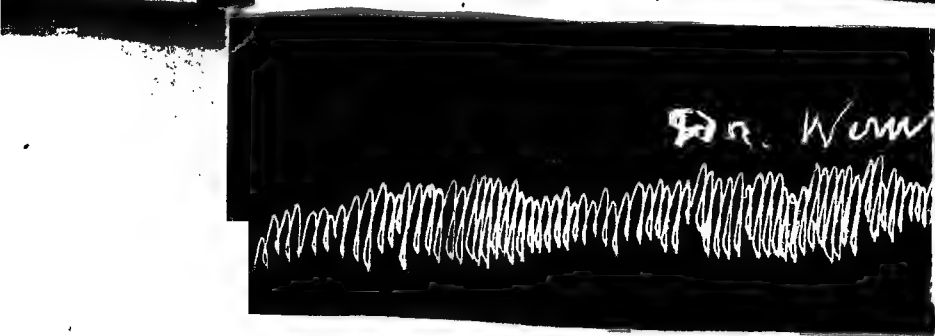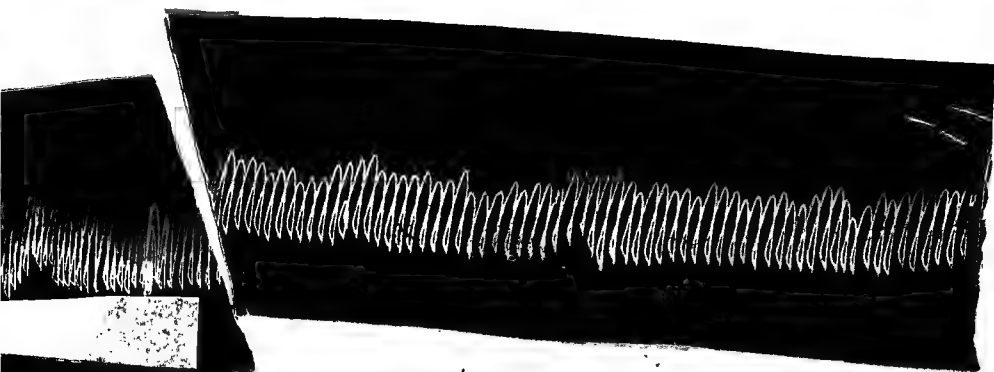
359. HILL AND BARNARD'S SPHYGMOMETER. Hold the sphygmometer in the vertical position, open the tap and press the edge of the rubber bag upon the table until the fluid rises to zero, then close the tap. By setting the fluid at zero before each reading, errors due to variations of atmospheric pressure and temperature are avoided. Having set the fluid at zero, press the rubber bag upon the radial artery until the maximal pulsation of the fluid is obtained. Read the pressure indicated on the scale at that point. For example : Suppose the pulsation is maximal between 110 and 120 mm. Hg. then the mean arterial pressure equals 115 mm. Hg. The normal average pressure in young men is 110–120 mm. Hg. in the standing and 100–110 mm. Hg. in the recumbent posture. The pulse frequency does not as a rule vary more than five beats per minute in the two positions. With this instrument the venous pressure can also be obtained. On the back of the hand or arm choose a vein free from anastomoses and press the sphygmometer upon the peripheral end of this. Then empty the vein centrally with the finger. Next gradually reduce the pressure of the sphygmometer and note the exact pressure at which the vein refills with blood.

360. THE PNEUMOGRAPH. Apply this apparatus to the thorax. Connect with a tambour and take a tracing on a revolving drum. Study the curves. Does expiration occupy a longer time than inspiration ? Compare with other members of the group.

Do not look at a tracing while it is being made, a person unconsciously attempts to regulate the breathing so that a perfect curve is made. Note the effect upon the curve of the following phenomena : squeeze suddenly a rubber bulb held in the right hand ; swallow a few mouthfuls of water ; hold the breath while you count ten ; speak a few words ; laugh. Have your co-worker indicate on the drum when each of these acts are performed. Use a time marker marking half seconds.

XXX
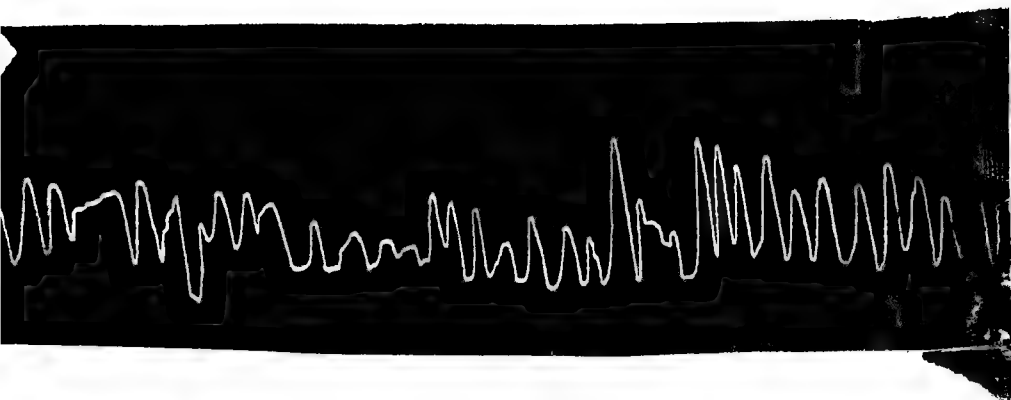
(The following exercise is taken, by arrangement with the publisher, from " An Introduction to Physiology," by W. T. Porter).

361. ARTIFICIAL SCHEME OF THE BLOOD CIRCULATION. This scheme illustrates the mechanics of the circulation in mammals. It consists of a pump (rubber bulb), a system of elastic tubes, and a peripheral resistance. The inlet and outlet tubes of the pump are furnished with valves which permit a flow in one direction only. The peripheral resistance is the friction which the liquid undergoes in flowing through the minute channels of a piece of bamboo (capillaries). To this should be added the slighter resistance due to friction in the rubber and glass tubes.
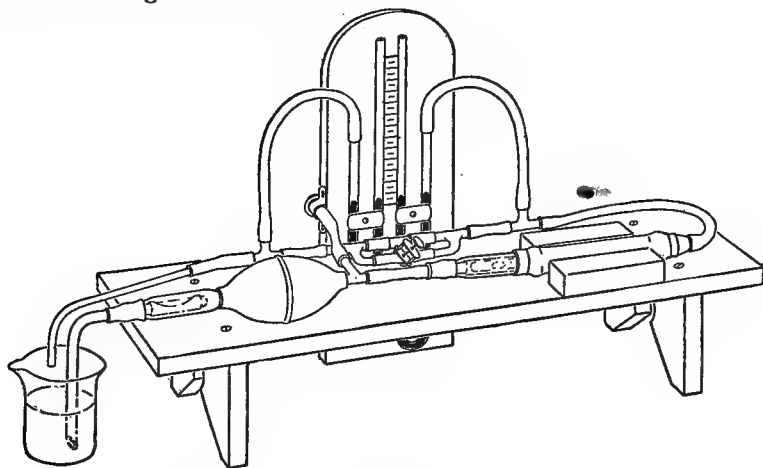
FIG. 27.

Diagram of Artificial Circulation Scheme (W. T. Porter). The bulb represents the ventricle; adjacent to it, on the left, the auriculo ventricular valves are represented. The cross branch on the right of the bulb is for measuring intraventricular pressure. A little to the right of this are the " aortic " valves. Immediately following is the arterial system with an arterial manometer inserted. Just in front of the upright portion of the apparatus is the capillary field with a side branch provided with a clamp for regulating peripheral resistance. From the capillaries to the beaker is the venous system, which is also provided with a manometer.

The pump represents the left ventricle ; the valve in the inlet and outlet tubes the mitral and aortic valves, respectvely ; the channels in the bamboo the resistance of the small

arteries and capillaries.   The tubes between the pump and
the resistance are the arteries ; those on the distal side of the
resistance are the veins.   The side branch around the bamboo
substitutes a wide channel for the narrow ones and is thus
equivalent to a dilatation of the vessels.   Between the pump
and the outlet valve is a side tube leading to a membrane
manometer which records the changes in the pressure (intra-
ventricular) within the pump (the loss in conveying the pres-
sure through the short, wide connecting tubes may be neg-
lected).   A mercury manometer is placed between the pump
and the capillary resistance to measure the arterial pressure,
and a second mercury manometer on the distal side of the
capillary resistance to measure the venous pressure.

FIG. 28

Diagram of valve used in the artificial circulation scheme (W. T. Porter).
The inner glass tube is provided with a jacket of rubber tubing.   The arrows
indicate the direction of the fluid.

The device used for the aortic valve consists of a small
glass tube which is fastened in a large glass tube by a collar
of rubber tubing.   The small glass tube is closed at one end.
One side is pierced with a valve hole.   The valve hole is
closed by a piece of rubber tubing which is drawn over the
small glass tube, and the middle portion of the rubber tubing
is cut away except over the hole.   During the stroke of the
pump the water enters the small glass tube under pressure,
lifts the rubber, escapes through the valve hole, and is carried
off by the large glass tube.   When the pressure in the small
glass tube is no longer as great as that in the surrounding
large glass tube, the rubber shuts the valve hole.   Backflow
is thereby prevented.   The mitral valve is similar to the
aortic, but the position of the small glass tube is reversed.

362.   THE CONVERSION OF AN INTERMITTENT FLOW
INTO A CONTINUOUS FLOW.   In a system of tubes with rigid
walls, a pump will force a fluid through the tubes in such a
way that the inflow and outflow will be equal and in the same
time.   The outflow ceases the instant the inflow ceases.

The same is practically true in a system of elastic tubes so short and wide that friction between the liquid and the walls causes practically no resistance to the flow. When the resistance is increased by narrowing the tubes, or by increasing their length, or in both these ways, not all the liquid received from the pump can pass by the resistance during the stroke of the pump,—the remainder must pass during the interval between one stroke and the next.

The portion which cannot pass during the stroke finds room between the pump and the resistance in the dilatation of the elastic containing vessels. As the pressure from the pump falls, the dilated elastic walls contract to their normal position and drive the liquid out of the tube past the resistance.

Open the side branch near the capillaries by unscrewing the pressure clip. See that the tubes are well filled with water. Make a single brief, gentle pressure on the bulb. Note (1) that practically all the liquid driven out by the stroke escapes through the side branch, in which the resistance is low, rather than through the high capillary resistance. (2) Only a portion of the liquid escapes during the stroke. (3) The portion which cannot escape by the resistance during the stroke finds space in a very evident dilatation of the tubes nearer the pump, *i. e.*, between the pump and the principal resistance. (4) The membrane manometer shows a sudden rise and fall indicating a sudden rise and fall in the intraventricular pressure. (5) Close observation shows that on the stroke of the pump the tubing just distal to the aortic valve begins to expand sooner than that farther away. Evidently the change of pressure produced by the stroke of the pump is transmitted from point to point through the liquid in the tubes. (6) The arterial manometer shows a sudden rise and fall. Observe that the rise is not simultaneous with the stroke of the pump, but begins an instant later. This interval is occupied by the transmission of the pressure change from the pump to the column of mercury, and in part by the time required to overcome the inertia of position of the mercury. The oscillations of the mercury following the primary rise and fall are due to inertia. (7) Observe the action of the valve (the arrangement consists of a glass tube, closed at one
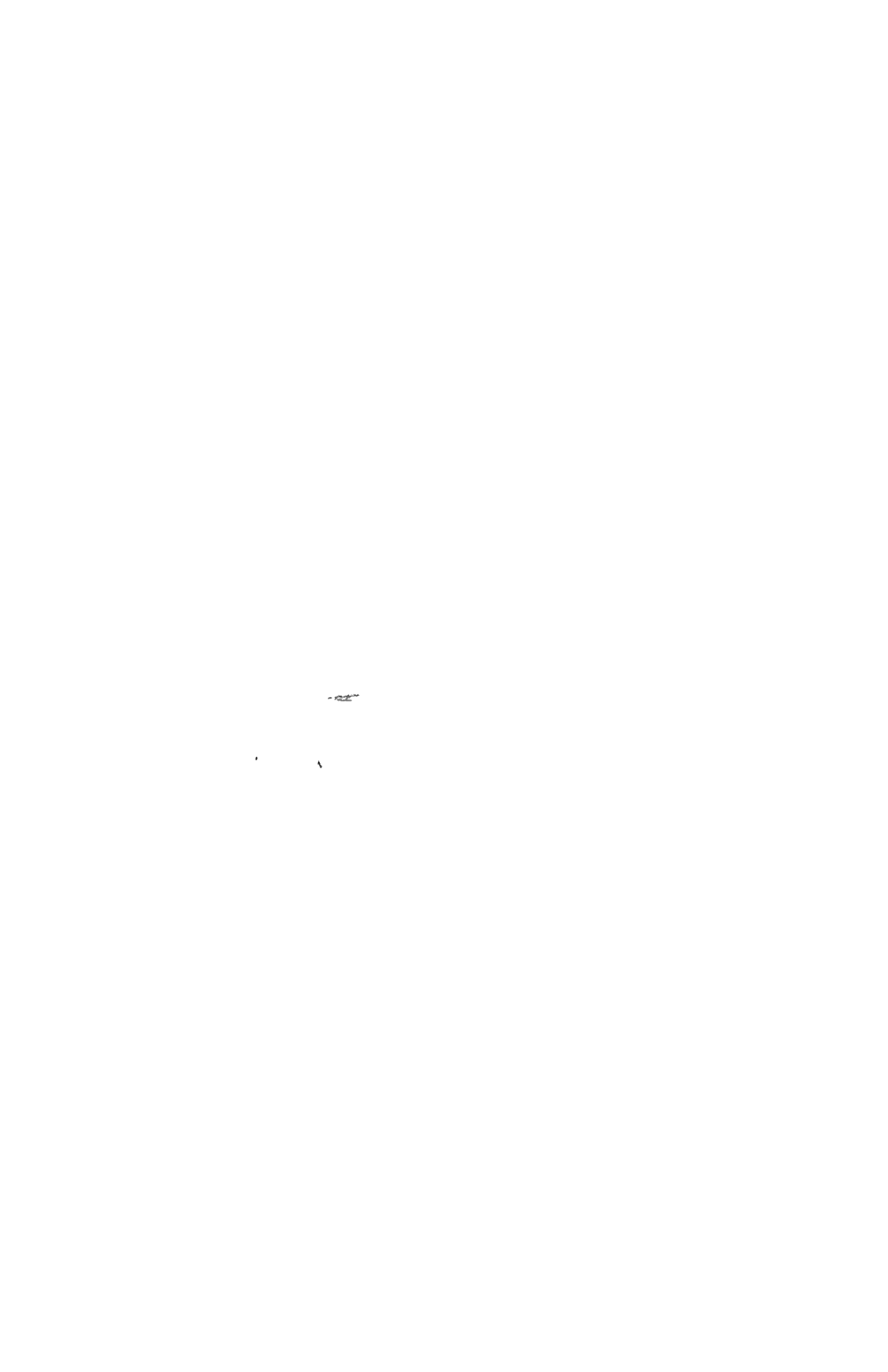
end, and pierced with a hole which is covered with a rubber flap fastened on both sides of the hole). (8) Place a finger on the "aorta" near the valve and note the pressure wave (pulse) as it passes along the vessel.

With the capillary side branch open as before compress the bulb rhythmically and gradually increase the frequency of the stroke. It will be found that at above twenty strokes to the minute the stream will be intermittent. As the interval between the strokes is shortened the liquid received from the pump in any one stroke cannot all escape through the resistance during the stroke and the succeeding interval. The next stroke comes before the outflow from the preceding stroke is finished, and the stream becomes remittent.

Still further increase the frequency of the stroke. A rate will be reached at which one-half the quantity received from the pump will pass by the resistance during the stroke of the pump and the remaining half will pass in the interval between that stroke and the next; the intermittent will be converted into a continuous flow.

Observe that the duration of the intervals is greater than the duration of the strokes of the pump. Thus the time during which the circulation is carried on by the energy stored by the pump in the elastic walls of the vessel is greater than the time during which it is carried on by the direct stroke of the pump.

Note that the arterial pressure remains low even after the stream becomes continuons. An increase in the frequency of the beat has little influence on the blood pressure where the peripheral resistance is very slight.

Close the side branch, so that the liquid must pass through a peripheral resistance. Compress the bulb at such rate that the outflow shall be continuous. The frequency required to make the flow continuous is now much less than when the peripheral resistance was low.

363. RELATION OF PERIPHERAL RESISTANCE TO BLOOD PRESSURE. Compress the bulb at a rate that will produce continuous outflow. With each successive stroke the portion of liquid unable to pass the resistance during the stroke and the succeeding interval is added to that left behind from preceding strokes. The arteries become more and more full.

The arterial manometer registers a higher and higher pressure. At length the pressure ceases to rise. The mercury remains at a mean level broken by a slight accession at each stroke. The pump now merely maintains the constant high arterial pressure. This pressure suffices to drive through the resistance during each stroke and the succeeding interval all the liquid received from the pump during the stroke.

The *venous pressure* remains very low. The capillary resistance (to which must be added the resistance of the smallest arteries) almost entirely exhausts the pressure in the arteries. Hence the sudden and profound difference observed between the arterial and the venous pressure. A second arterial manometer placed near the aorta will show that the loss of pressure between the ventricle and the smallest arteries is relatively slight. The pulse is absent on the venous side of the resistance.

364. CHANGES IN THE STROKE OF THE PUMP ; INHIBITION OF THE VENTRICLE. While the arterial pressure in the artificial scheme is at a good height (about 120 mm. Hg.) arrest the ventricular stroke, (comparable to cardiac inhibition through the vagus in animals). So soon as the ventricle ceases to beat, the less distended arteries will empty themselves through the peripheral resistance, and the arterial manometer will show a continuous fall in blood pressure. Resume the ventricular beats. The mercury in the arterial manometer will rise in large leaps, corresponding to the ease with which the early strokes of the pump distend the lax arteries (the inertia of the mercury somewhat exaggerates the rise at each stroke). As the blood pressure rises, however, the excursion of the mercury for each ventricular stroke becomes less and less, corresponding to the smaller and smaller difference between the pressure in the arteries and the maximum pressure within the ventricle, until at length equilibrium is restored between the peripheral resistance and the force and frequency of the ventricular beat.

365. THE OPENING AND CLOSING OF THE VALVES. Secure a high arterial pressure (120 mm. Hg.) in the artificial scheme. Now greatly slow each ventricular beat and at once observe closely the action of the valves. It will be seen that the mitral valve closes as soon as the ventricle begins to con-

tract, but the aortic valve does not open until the intraventricular pressure has risen above that in the aorta. Time is required for this rise in the pressure in the ventricle. During this period both mitral and aortic valves are closed. When the ventricle begins to relax, the intraventricular pressure speedily falls below that in the aorta, and the aortic valve shuts, but the intraventricular pressure normally must fall at least 100 mm. Hg. farther before it shall be lower than that in the auricle. During this fall all the heart valves are again closed ; the aortic valves are already shut, and the mitral not yet open.

366. THE PERIOD OF OUTFLOW FROM THE VENTRICLE. Tie a rubber membrane over the small thistle-tube of the sphygmograph and cement a bone button in the centre. Prepare a second receiving tambour in the same way. Bring the writing points of the recording tambours into the same vertical line against the smoked drum. Let the drum revolve at its fastest speed. Place the button of one receiving tambour on the aorta, the other on the membrane of the tube which records the intraventricular pressure. Let the ventricle pump with the usual force and frequency. When the two curves have been written, stop the clock-work and turn back the drum until the point of the lever recording the ventricular pressure lies at the exact beginning of the upstroke in the aortic pulse curve. Cause each lever to write an ordinate on the stationary drum. These ordinates will indicate synchronous points and will mark the beginning of the " out-flow " period. Now turn the drum until the point of the aortic lever lies beneath the notch seen in the down-stroke of the pulse curve (the dicrotic notch). Describe synchronous ordinates. It is known that the dicrotic notch in the aortic pulse curve corresponds closely to the moment of closure of the aortic valves. It marks therefore the end of the outflow period. Note that this point is reached soon after the ventricle begins to relax. Thus the period during which the intraventricular pressure is higher than the pressure in the aorta embraces part of the relaxation as well as part of the contraction of the ventricle. It includes approximately the highest third of the intraventricular pressure curve.

Observe also the considerable interval between the begin-

ning of ventricular contraction and the opening of the aortic valve, as shown by the upstroke in the pulse curve consequent upon the entrance of liquid into the aorta.

XXXI

367. BLOOD PRESSURE IN THE FROG. Curarize a frog lightly, and expose the heart with the aortae leading off from it. Get ready a fine cannula with a short piece of rubber tubing attached. Fill the tubing and cannula with a 1 per cent solution of Sodium Carbonate and close the end of the tube with a clamp. Dissect out one of the aortae and tie a ligature around it as far as possible from the heart. Pass a second ligature around the same aorta, without tying, nearer to the heart. Lift the aorta with the second ligature and with a pair of sharp pointed scissors make a slight incision in the vessel and introduce the cannula into this incision and tie it with the second ligature. Fill the proximal end of the manometer with a 1 per cent solution of Sodium Carbonate seeing that all air is excluded, so that when the tubing is attached to the manometer, there will be a continuous volume of the Sodium Carbonate solution from the cannula to the mercury of the manometer. Before attaching the tubing to the manometer, clamp the aorta or have your co-worker compress it carefully with a pair of forceps. Place the frog-board on a wooden stand, so as to bring the heart to a slightly higher level than the level of the mercury in the manometer. Bring the writing point of the lever of the manometer against a smoked drum and revolve the drum once so as to record a line of atmospheric pressure.

After the cannula in the aorta, with its tube has been attached to the manometer, remove the clamp of forceps from the aorta and allow the blood from the heart to pump against the Sodium Carbonate and mercury in the manometer. The columns of mercury in the proximal and distal tubes will be no longer at approximately the same level. The mercury in the proximal tube will fall slightly and will rise correspondingly in the distal tube. Note that with each beat of the ventricle the column rises a short distance above the mean

level and sinks again. Get a tracing of this blood pressure curve upon a very slowly revolving drum. The actual pressure, in millimeters of mercury, is obtained by multiplying the mean height of the curve, above the atmospheric line, by two.

368. STANNIUS'S EXPERIMENTS ON THE FROG'S HEART. Some of the early and important experiments relating to the beat of the frog's heart were performed by Stannius, and bear his name.

If the sinus venosus is separated from the rest of the heart by a ligature of thread passed under the aorta and drawn tightly around the sinus at its junction with the auricle, the sinus venosus continues to pulsate, but the auricles and ventricle are quiescent. If the auricles are now separated from the ventricle by a thread ligature tied around the auriculo-ventricular groove, the auricles remain motionless, but the ventricle begins to beat, so that the sinus venosus and ventricle are pulsating, but with a different rhythm, while the auricles are at rest. The rate of the ventricular beat is usually much slower than that of the sinus.
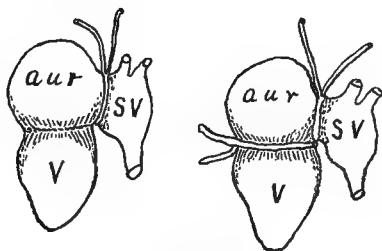
FIG. 29

Fig. 29. Aur, auricle ; V, ventricle ; S V, Sinus Venosus. The figure to the left shows the application of the ligature between the sinus and the auricle. In the figure to the right there is shown the second ligature between the auricle and ventricle.

The quiescence of the auricles and ventricle, in the first case, has been supposed to show that the motor centers for the entire heart reside in the sinus, and that from them the motor impulses originate which keep up the rhythmical pulsations of the organ, But the fact that the ventricle begins to pulsate on its own account, as in the second case, when separated

by another ligature from the auricles, seems to show that it also contains motor centers. The hypothesis has been advanced that both sinus venosus and ventricle contain motor centers, while the auricles contain inhibitory centers.

So long as the auricles are in connection, both with the sinus venosus and the ventricle, the motor centers in the latter two parts are supposed to be sufficiently powerful to overcome the resistance offered by the inhibitory centers, and thus the cardiac rhythm is maintained. When the motor centers of the sinus are removed, the inhibitory centers of the auricle are supposed to be so powerful as to keep both it and the ventricle in a state of rest.

369. CARDIAC DELAY OR LATENT PERIOD OF CARDIAC MUSCLE. In the case of skeletal muscle, the muscle is at rest and a stimulus excites it to contraction ; cardiac muscle has the power of contracting rhythmically ; it will therefore be necessary to stop the heartbeat by the application of a " Stannius " ligature.

Arrange the apparatus for single induced shocks, and include in the primary circuit a signal magnet to mark the exact time the stimulus is applied. Use also a time-marker recording in half seconds.

After exposing the heart apply the " Stannius ligature " to stop the beat. Attach the apex of the ventricle to the heart lever. Arrange the three levers, heart, signal magnet and time marker so that their writing points will all be exactly in the same vertical line. Let the drum revolve. Stimulate the ventricle with a single induced shock. When the circuit is made or broken the lever of the signal magnet will immediately respond and shortly after the heart lever will also respond. The interval represents the " latent period " and may be about half a second, depending upon temperature and other conditions.

Stimulate an auricle in the same way and note the longer " delay ;" the wave of contraction travelling slowly and delaying at the groove.

Compare the cardiac latent period with the latent period of skeletal muscle.

370. MAXIMUM CONTRACTIONS ONLY. Find the weakest stimulus that will cause contraction of the ventricle. Increase

the strength of the stimulus but do not stimulate more than once in ten seconds, otherwise "staircase" contractions may result. The force of the ventricular contraction will remain the same in spite of the stronger stimulus. If the heart is capable of responding at all it will, in each case, give a maximum contraction. Stimulate either auricle in the same way and note the result.

371. STAIRCASE CONTRACTIONS OF THE HEART. Apply the first Stannius ligature over the sino-auricular groove. Connect the apex of the heart with the heart lever. Record on a slowly moving drum. Stimulate the quiescent heart with single induction shocks at intervals of five seconds. Notice that the second beat is higher than the first, the third than the second and so on until a maximum beat is obtained. This is the "staircase" of Bowditch.

372. LOCATION OF MOTOR CENTERS IN THE FROG'S HEART. Dissect out the entire heart of a frog, and note that it continues to beat. Cut the heart vertically into three pieces, so that the middle portion will contain the auricular septum, in which lie the ganglionic cells. This portion continues to beat while the right and left lateral parts do not beat spontaneously, but will respond with a single contraction if stimulated.

373. EFFECT OF TEMPERATURE UPON THE HEART BEAT. Leaving the heart in its usual position insert a glass tube into the œsophagus and allow it to project through the stomach. Pass water at different temperatures through the tube and note the number of beats in each case.

374. BERNSTEIN'S EXPERIMENT. ISOLATED APEX.—Tie a ligature around the ventricle about half way between its apex and base, or compress it with a clamp or pair of forceps, the object being to destroy physiological continuity but preserving anatomical connection. Remove the ligature. The physiologically isolated apex does not contract. This would seem to indicate that the adult heart muscle is incapable of spontaneous rhythmical contraction. If the bulbus arteriosus is compressed, the pressure of blood in the ventricle rises and is usually sufficient to stimulate the apex strongly enough to start it beating again. Remove the ligature and apply the pressure to the bulbus and note the effects.

375. INTRACARDIAC INHIBITORY CENTER IN THE FROG. Expose the heart of a frog, divide the frenum and tilt the heart upward to expose the whitish V-shaped crescent between the sinus venosus and right auricle. Stimulate the crescent, using fine electrodes, with an interrupted current ; if the current is sufficiently strong, the auricles and ventricle, after a brief delay, will cease to beat for a time, but they begin beating again even in spite of continued stimulation. Stimulate the auricles ; there is no inhibition.

Connect the apex of the ventricle with the heart lever. Use a signal magnet marking seconds, in the primary circuit. Its lever will vibrate when the circuit is closed. Arrange so that its writing point will write immediately under and in the same vertical plane as the writing point of the heart lever. Get a tracing of the normal beat, then stimulate the crescent for one or two seconds as before. Inhibition results. After a pause the beat begins again, the contraction passing as a wave from the sinus, through the auricles to the ventricle.

Stimulate the auricles. Note any effect upon the tracing. (During inhibition the sinus beats, but the auricles and ventricle do not, because the excitability is so lowered that they do not propagate the excitatory process.

Stimulate the ventricle mechanically, the heart beats in the reverse order from ventricle through auricles to sinus.

Apply a few drops of atropine solution to the heart and again stimulate the crescent. There is no inhibitory effect as the atropine paralyzes the inhibitory fibers.

376. FORM AND VOLUME OF A CONTRACTING MUSCLE. Dissect out the gastrocnemius muscle of a frog. Connect the hooked electrodes at each end of the volume tube with the muscle. The tube is to be filled with saline solution which has been boiled and allowed to cool down to the temperature of the room. Replace the stopper in the tube in such a way that all air bubbles shall be excluded. The height of the water in the capillary tube may be adjusted to the proper level by moving the glass rod in the stopper in or out. Connect the electrodes of the volume tube with the secondary coil and, using a single induction current, send a maximal break shock into the muscle. Note very carefully the level of the water in the capillary tube before, during and after the contraction of the limb. Does the level of the water in the capillary tube change ?

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